



HAL
open science

Subthalamic nucleus stimulation impairs motivation: Implication for apathy in Parkinson's disease

Yvan Vachez, Carole Carcenac, Robin Magnard, Lydia Kerkerian-le Goff,
Pascal Salin, Marc Savasta, Sebastien Carnicella, Sabrina Boulet

► **To cite this version:**

Yvan Vachez, Carole Carcenac, Robin Magnard, Lydia Kerkerian-le Goff, Pascal Salin, et al.. Subthalamic nucleus stimulation impairs motivation: Implication for apathy in Parkinson's disease. *Movement Disorders*, 2020, 10.1002/mds.27953 . hal-02519001

HAL Id: hal-02519001

<https://hal.science/hal-02519001>

Submitted on 17 Nov 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**Subthalamic nucleus stimulation impairs motivation:
implication for apathy in Parkinson's disease**

Journal:	<i>Movement Disorders</i>
Manuscript ID	MDS-19-0389.R3
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Vachez, Yvan; Inserm, Grenoble Institute of Neuroscience - Dynamic and Pathophysiology of Basal Ganglia; Grenoble Institut des Neurosciences Carcenac, Carole; Grenoble Institut des Neurosciences Magnard, Robin; Grenoble Institut des Neurosciences Kerkerian, Lydia; IBDM Salin, Pascal; IBDM Savasta, Marc; Grenoble Institut des Neurosciences Carnicella, Sebastien; Grenoble Institut des Neurosciences Boulet, sabrina; Grenoble Institut des Neurosciences
Keywords:	Deep brain stimulation, Neuropsychiatry, Parkinson's disease, Apathy, Dopamine

SCHOLARONE™
Manuscripts

1
2
3 **Subthalamic nucleus stimulation impairs motivation: implication for apathy in**
4
5 **Parkinson's disease**
6
7
8

9 Yvan Vachez, PhD^{1,2}, Carole Carcenac, PhD^{1,2}, Robin Magnard, PhD^{1,2}, Lydia Kerkerian -Le Goff,
10
11 PhD³, Pascal Salin, PhD³, Marc Savasta, PhD^{1,2}, Sebastien Carnicella, PhD^{1,2} and Sabrina Boulet,
12
13
14 PhD^{1,2}
15

16 **Affiliations :**

17 ¹Inserm, U1216, F- 38000 Grenoble, France.

18 ²Univ. Grenoble Alpes, Grenoble Institut des Neurosciences, GIN, F-38000 Grenoble, France.

19 ³Aix Marseille Univ, CNRS, IBDM, Marseille, France.
20
21
22
23
24
25

26 **Corresponding authors:**

27 Dr Sabrina Boulet and Dr Marc Savasta

28
29
30 INSERM U1216, Grenoble Institute of Neuroscience, Group "Pathophysiology of Motivation",
31
32 Grenoble Université - Site Santé La Tronche - BP 170, 38042 Grenoble, France. *Phone:* 33 4
33
34 56 52 06 70;
35

36
37 *Email addresses:* [Sabrina.boulet@univ-grenoble-alpes.fr](mailto: Sabrina.boulet@univ-grenoble-alpes.fr) ; [marc.savasta@inserm.fr](mailto: marc.savasta@inserm.fr)
38
39
40

41 **Running title:** STN-DBS and Apathy in Parkinson's Disease
42
43
44

45 **Relevant conflicts of interest/financial disclosures:** Nothing to report.
46
47
48
49

50 Number of characters in the title: 130

51 Number of characters in the running title: 41

52 Number of words in the Abstract: 250

53
54 Number of words in the body of the manuscript: 4387

55
56
57 Number of figures: 5
58
59
60

STN-DBS and Apathy in Parkinson's Disease

ABSTRACT

Background: Apathy is one of the most disabling neuropsychiatric symptoms in Parkinson's disease (PD) patients, and has a higher prevalence in patients under subthalamic nucleus deep brain stimulation (STN-DBS). Indeed, despite its effectiveness for alleviating PD motor symptoms, its neuropsychiatric repercussion has not been fully uncovered yet. Because it can be alleviated by dopaminergic therapies, especially D_2 and D_3 dopaminergic receptor (D_2R/D_3R) agonists, the commonest explanation proposed for apathy after STN-DBS is a too strong reduction of dopaminergic treatments.

Objectives: Whether or not STN-DBS can induce apathetic behaviors remains an important matter of concern. We aimed at unambiguously addressing this question of the motivational effects of chronic STN-DBS.

Methods: We longitudinally assessed the motivational effects of chronic STN-DBS, by using innovative wireless micro-stimulators allowing continuous stimulation of STN in freely moving rats, and a pharmacological therapeutic approach.

Results: We showed for the first time that STN-DBS induces a motivational deficit in naïve rats and intensifies those existing in a rodent model of PD neuropsychiatric symptoms. As reported from clinical studies, this loss of motivation was fully reversed by chronic treatment with pramipexole, a D_2R/D_3R agonist.

Conclusion: Taken together, these data provide experimental evidence that chronic STN-DBS by itself can induce a loss of motivation, reminiscent of apathy, independently of the dopaminergic neurodegenerative process or reduction of dopamine replacement therapy, presumably reflecting a dopaminergic driven deficit. Therefore, our data help to clarify and reconcile conflicting clinical observations by highlighting some of the mechanisms of the neuropsychiatric side-effects induced by chronic STN-DBS.

STN-DBS and Apathy in Parkinson's Disease

1 Introduction

2 Subthalamic nucleus deep brain stimulation (STN-DBS) is a neurosurgical treatment
3 that efficiently alleviates the motor symptoms of Parkinson's disease (PD).¹ However, a
4 plethora of psychiatric manifestations and cognitive deficits have been recently identified as
5 an integral part of the clinical picture of the disease and STN-DBS has been suggested to
6 influence these symptoms, for better or for worse.^{2, 3} Apathy, which can be simplistically
7 defined as a loss of motivation or a reduction in goal-directed behaviors accompanied by loss
8 of emotions and flattening of affect,⁴⁻⁶ is the most frequently observed non-motor
9 complication of PD and deeply contributes to worsen the patient's quality of life^{2, 5, 7-12}.
10 Importantly, apathy has been reported to occur, or be exacerbated, in some patients under
11 STN-DBS, as blunted affects^{8-10, 13-16}. Yet, the clear repercussion of STN-DBS on apathy
12 remains to be elucidated.

13 Because dopaminergic replacement therapy (DRT) is reduced during STN-DBS,
14 apathy in patients under STN-DBS is commonly attributed to the resurgence of pre-existing
15 symptoms revealed by DRT reduction or withdrawal.¹⁷ This assumption is also supported by
16 1) the alleviation of apathy after STN-DBS by dopaminergic agonists, especially those
17 targeting the D2 and D3 DA receptors (D₂R and D₃R)^{18, 19} and 2) functional imaging studies
18 in PD patients^{9, 17} and some preclinical data indicating that at least some forms of apathy are
19 related to the degenerative process and DA loss.²⁰

20 However, in several studies, the occurrence of apathy in patients under STN-DBS
21 was not correlated with the reduction of DRT but with DBS-induced changes in glucose
22 metabolism within the associative and limbic circuitry^{15, 21, 22} or with incorrect location of
23 electrodes in the associative or limbic part of the STN.²³ Thus, apathy in patients under STN-
24 DBS was also proposed to be a major side-effect of STN-DBS itself.^{15, 21-23} There is now an
25 abundance of experimental and clinical studies demonstrating that 1) the STN is involved in
26 reward and motivational processes,²⁴⁻³¹ 2) manipulating the STN can modify motivational
27 behaviors³²⁻³⁷ and 3) STN-DBS can alleviate dopamine dysregulation syndrome in some PD

STN-DBS and Apathy in Parkinson's Disease

1 patients^{14, 38, 39} and be a conceivable approach to treat addictive disorders⁴⁰⁻⁴⁵. Yet, a
2 possible direct impact of STN-DBS on motivation in PD is not unanimously supported.

3 This lack of consensus concerning the origin of apathy in patients under STN-DBS is
4 a critical issue. Whereas the prevalence of apathy before STN-DBS is about 25%^{13, 46, 47}, this
5 percentage ranges from 8 % to 60 % during STN-DBS^{14, 17, 21, 48}, according to the diagnostic
6 approach used, whether established according to cut-off scores on severity scales,
7 instruments rated by caregivers, or clinical diagnostic criteria⁴⁷. Thus, it considerably
8 compromises the benefits of STN-DBS on motor symptoms.⁵ Investigating this question in
9 patients is difficult because it is impossible to avoid the impact of the progressive process of
10 degeneration and reduction of DRT during STN-DBS. In addition, the animal studies that
11 have sought to explore the limbic and mood effects of STN-DBS may not have combined
12 appropriate behavioral approaches to assess motivation with bilateral and continuous STN-
13 DBS^{41, 49, 50} as clinically applied most of the time in studies reporting on apathy^{5, 15, 19, 21, 51-53}.

14 In the present study, we explored a potential effect of bilateral STN-DBS on
15 motivation, by using a wireless micro-stimulation system enabling chronic continuous
16 stimulation in rats during several weeks⁵⁴. We first investigated the consequences of bilateral
17 chronic STN-DBS in naïve rats and then in a preclinical model of neuropsychiatric symptoms
18 related to PD that we have developed, using bilateral but partial denervation of the dorsal
19 striatum (DS) by 6-hydroxydopamine (6-OHDA) lesion of SNc.^{20, 55} Because D₂R and D₃R
20 agonists, such as pramipexole (PPX), can alleviate pre and post STN-DBS apathy in PD
21 patients^{18, 19, 56} as well as in preclinical models^{20, 57} and because we reported that STN-DBS
22 reduces the level of D₂R and D₃R in the nucleus accumbens (NAc) of rats,⁵⁸ our working
23 hypothesis was that post-STN-DBS apathy may be due to an alteration of DA transmission
24 induced by STN-DBS itself. Thus, we also investigated the effect of STN-DBS with or without
25 chronic treatment with PPX.

1 **Materials and Methods**

2 **Animals**

3 Experiments were performed on adult male Sprague-Dawley rats (Janvier, Le Genest-Saint-
4 Isle, France) weighing approximately 350g (8 weeks old) at the time of surgery. Animals
5 were individually housed under standard laboratory conditions (12 h light/dark cycle, with
6 lights on at 7 am) with food and water available *ad libitum* during all the experimental
7 procedures. Protocols used complied with the European Union 2010 Animal Welfare Act and
8 the French directive 2010/63.

10 **Bilateral 6-OHDA lesions, bilateral implantation of electrodes and deep brain 11 stimulation of subthalamic nucleus**

12 See supplemental informations

14 **Experimental design**

15 After self-administration training and both surgeries, rats were subjected to a sequence of
16 behavioral tests, with one resting day between the different tests (Figure 1A). In each
17 experiment, all conditions were counter-balanced among the different test chambers and
18 each apparatus was thoroughly cleaned after each trial or session.

19 Rats were trained to self-administer a 2.5% sucrose solution before and after SNc lesion and
20 electrodes implantation (Only the self-administration after electrode implantation is
21 represented). After 10 to 15 days, stable performances were obtained (less than 20%
22 performance variation over three consecutive sessions) and STN-DBS was turned ON.
23 Pharmacological procedures were applied after a new stabilization period: PPX (Sigma, 0.3
24 mg.kg⁻¹ in 0.9% NaCl, 1 ml/kg) or vehicle was administered (sub-cutaneous) twice a day, 3h
25 before the beginning of behavioral tests (i.e. injection at 7 am; test at 10 am) for and then at
26 5 pm, during 20 days. This protocol, known to increase the expression of D₂R and D₃R,⁵⁹
27 was chosen to explore the chronic effects of PPX.⁶⁰ STN-DBS and PPX treatment were

STN-DBS and Apathy in Parkinson's Disease

1
2
3 1 uninterrupted until rat euthanasia. After several days of sucrose self-administration, rats were
4
5 2 submitted to a two-bottle choice procedure, as well as to a stepping test and
6
7 3 locomotor/ambulatory activity evaluation in an open area⁵⁵ (Figure 1A). At the end of the
8
9 4 experiment, rats were euthanized and brains were processed for histological control of lesion
10
11 5 and implantations.
12
13 6

15 7 See supplemental data for full description of behavioral procedures, quantification of the
16
17 8 extent of the striatal DA denervation and control of electrode implantation.
18
19 9

Data and statistical analysis

21
22 10
23
24 11 Data are shown as means \pm SEM and were analyzed by one or two-way ANOVAs, repeated
25
26 12 or not as specified in Results. Concerning operant sucrose self-administration, the different
27
28 13 experimental periods (Pre-STN-DBS, STN-DBS and STN-DBS + PPX) were analyzed
29
30 14 independently by distinct repeated measure ANOVAs for figures 2A and 4A. When indicated,
31
32 15 post hoc analyses were carried out with the Student Newman-Keuls procedure.
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

STN-DBS and Apathy in Parkinson's Disease

1 Results**2 *Histological controls***

3 Figure 1B provides the different electrode positions in left and right STN for the
4 stimulated animals. Figures 1C and D illustrate two examples of the position of an electrode
5 tip within the STN, stimulated respectively with the lowest (100 μ A) and the highest (225 μ A)
6 intensity used in the study. See also supplemental figure 1 for additional examples of STN
7 stimulated at 100 μ A or 225 μ A, still unaltered after chronic stimulation.

8 Bilateral lesion of SNc (Figure 1E, percent of TH-IR loss, left SNc: 73 ± 5 ; right SNc:
9 74 ± 5) was obtained by 6-OHDA injection. The injection produced an important denervation
10 of the dorsal striatum in its lateral portion (Figure 1F, left dorsal striatum: 68 ± 5 ; right dorsal
11 striatum: 74 ± 6), along its rostro-caudal extent as revealed by decreased tyrosine
12 hydroxylase immunoreactivity. As the lesion has been shown to specifically affect SNc,
13 barely impacting VTA (Figure 1G, left VTA: 11 ± 8 ; right VTA: 20 ± 4 ; Two way ANOVA,
14 Structure x Lesion interaction: $F_{1,22} = 7.654$, $p < .0151$), NAc was almost totally preserved
15 from denervation (Figure 1H, left NAc: 21 ± 3 ; right NAc: 24 ± 4 ; Two way ANOVA, Structure
16 x Lesion interaction: $F_{1,22} = 125.758$, $p < .001$).

18 *STN-DBS induces a motivational deficit in normal rats that is reversed by the D₂R/D₃R*
19 *agonist pramipexole*

20 Before STN-DBS was switched ON, rats were trained for 10 days in the operant task
21 (only the last 3 days of training are represented in figure 2A). Groups were formed to have
22 equivalent performance levels (Figure 2A, Pre STN-DBS period, repeated measure ANOVA,
23 Session x STN-DBS x PPX interaction, $F_{2,122} = 1.334$, $p = .2672$). From day one of
24 stimulation, STN-DBS induced a dramatic decrease of about 40% in instrumental responding
25 for the sucrose solution in both stimulated groups before pharmacological treatment (STN-
26 DBS + Veh and STN-DBS + PPX) as compared with the pre STN-DBS levels (Figure 2A,
27 STN-DBS period, repeated measure ANOVA, STN-DBS effect: $F_{1,54} = 9.615$, $p = .0031$;

STN-DBS and Apathy in Parkinson's Disease

1
2
3 1 Figure S2A, two way ANOVA, STN-DBS effect: $F_{1,42} = 10.480, p = .002$). PPX completely
4
5 2 rescued the self-administration performances of stimulated rats from the second day of
6
7 3 treatment, without bringing performances superior to baseline or to Control + Veh rat levels.
8
9 4 (Figure 2A, STN-DBS + PPX period, repeated measure ANOVA, STN-DBS x PPX
10
11 5 interaction: $F_{1,58} = 4.658, p = .0351$; Session x PPX interaction: $F_{6,348} = 2.412, p = .0269$).
12
13 6 Both STN-DBS and PPX effects remained stable until the end of self-administration
14
15 7 experimentation (Figure 2B, two ways ANOVA, STN-DBS x PPX interaction: $F_{1,64} = 7.178, p$
16
17 8 = .009). In order to determine whether the decrease of operant performances induced by
18
19 9 STN-DBS was due to impairment in the consummatory or preparatory components of
20
21 10 motivated behaviors, we evaluated the sensitivity to the motivational properties of sucrose
22
23 11 (consummatory behavior) in a two-bottle choice procedure. The preference for the sucrose
24
25 12 solution that was used in the operant self-administration experiment over water was high and
26
27 13 not altered by STN-DBS or PPX (Figure 2C, two way ANOVA, STN-DBS x PPX interaction:
28
29 14 $F_{1,61} = .966, p = .330$). Therefore, the self-administration paradigm and the sucrose
30
31 15 preference test indicate that STN-DBS affected the preparatory component of motivated
32
33 16 behaviors during the operant task and that PPX specifically corrected this impairment.

34
35
36 17 In addition, STN-DBS-induced preparatory component deficit and subsequent self-
37
38 18 administration deficiency cannot be attributed to motor impairment, because STN-DBS did
39
40 19 not induce any significant locomotor change during the first 20 minutes of the open area test,
41
42 20 corresponding to the exploratory phase when locomotor activity is high, facilitating detection
43
44 21 of motor deficit (Figure 3A, two way ANOVA, 0-20 min: $F_{1,48} = .513; p = .477$). Exploration
45
46 22 and locomotor activity then progressively decreased to a basal level during the last 2 periods
47
48 23 (20 to 60 min). Although it did not reach significance, STN-DBS tended to reduce ambulatory
49
50 24 activity during this phase. (Figure 3A, STN-DBS effect, Two way ANOVA, 20-40 min: $F_{1,48} =$
51
52 25 $3.311, p = .075$; 40-60 min: $F_{1,48} = 3.525, p = .067$) This effect of STN-DBS is unlikely to
53
54 26 reflect a motor deficit but rather a decrease in general behavioral activity, because
55
56 27 ambulatory speed during this test phase was not changed by the stimulation (Figure 3B,
57
58 28 STN-DBS effect, Two way ANOVA, $F_{1,48} = .196, p = .660$). Moreover, fine motor skills of left

STN-DBS and Apathy in Parkinson's Disease

1 and right limbs assessed using a stepping test were not affected by the stimulation,
2 confirming the absence of any motor deficit induced by chronic and bilateral STN-DBS
3 (Figure 3C). As STN-DBS, PPX had no impact on fine motor skills (Figure 3C, two way
4 ANOVA, STN-DBS x PPX interaction: left to right moves, left limb: $F_{1,55} = .110$, $p = .742$; right
5 limb $F_{1,55} = .000442$, $p = .983$; right to left moves, left limb: $F_{1,55} = .0135$, $p = .908$; right limb:
6 $F_{1,55} = .0557$, $p = .814$), but caused a significant increase in locomotor activity in the open
7 area test (Figure 3A, PPX effect, Two way ANOVA, 20-40 min: $F_{1,48} = 5.686$, $p = .021$; 40-60
8 min: $F_{1,48} = 5.047$, $p = .030$). Interestingly, the combined effect of the behavioral hypoactivity
9 induced by STN-DBS and hyperactivity due to PPX resulted in normalization of the general
10 activity during the last 2 phases of the open area test (Figure 3A).

11 Taken together, these results suggest that STN-DBS induced a clear motivational
12 deficit that was alleviated by the D_2R/D_3R agonist, reminiscent of apathy in PD.

13
14 ***STN-DBS exacerbates motivational deficit in a rodent model of PD apathetic***
15 ***symptoms and affective disorders***

16 We sought to confirm whether STN-DBS would also affect motivation in a
17 pathological context. We used a rodent model of PD that we developed⁵⁵ and that exhibits
18 strong motivational deficits, without locomotor alterations.

19 Before STN-DBS was switched-ON, rats were trained again for 10 days in the
20 operant task (only the last 3 days of training are represented in figure 4A). All groups
21 obtained equivalent performance levels (Figure 4A, 3 last days of Pre STN-DBS period,
22 repeated measure ANOVA, Session x STN-DBS x PPX interaction, $F_{2,32} = 1.088$, $p = .3490$).
23 As previously demonstrated,⁵⁵ 6-OHDA rats showed a marked and significant decrease in
24 self-administration of sucrose as compared to non-lesioned animals (Figure S2A, two way
25 ANOVA, Lesion effect: $F_{1,42} = 26.592$, $p = < .001$; Figure S2B, two way ANOVA, Lesion
26 effect: $F_{1,50} = 26.368$, $p = < .001$) which was not reversed by PPX treatment alone (Figure
27 S2B, two way ANOVA, PPX effect: $F_{1,50} = .150$, $p = .701$). Despite this important instrumental
28 deficit induced by the lesion, STN-DBS was still able to reduce operant behavior by about

STN-DBS and Apathy in Parkinson's Disease

1
2
3 1 50% from day one of stimulation (Figure 4A, STN-DBS period, repeated measure ANOVA,
4
5 2 STN-DBS effect $F_{1,15} = 4.249$, $p = .0571$), as observed in non-lesioned/control stimulated rats
6
7 3 (Figure S2A, two way ANOVA, STN-DBS effect: $F_{1,42} = 10.48$, $p = .002$ and no STN-DBS x
8
9 4 Lesion interaction: $F_{1,42} = 1,112$, $p = .298$). As for non-lesioned animals, PPX completely
10
11 5 alleviated this STN-DBS-induced deficit from the second day of treatment (STN-DBS + PPX
12
13 6 period, repeated measure ANOVA, STN-DBS x PPX x Session interaction effect $F_{9,108} =$
14
15 7 2.712 , $p = .0069$). Both STN-DBS and PPX effects remained stable until the end of the
16
17 8 experiment (Figure 4B, two way ANOVA, STN-DBS x PPX interaction: $F_{1,16} = .826$, $p = .377$).
18
19 9 We also assessed the consummatory components of motivated behaviors, with the two-
20
21 10 bottle choice procedure and we found that neither STN-DBS nor PPX modified the
22
23 11 preference for sucrose (Figure 4C, two way ANOVA, STN-DBS x PPX interaction: $F_{1,20} =$
24
25 12 $.341$, $p = .567$).

26
27
28 13 PPX or STN-DBS tended to reduce ambulatory activity in the open area test while
29
30 14 when they were combined, on average they promoted a high level of activity but with high
31
32 15 variability (Figure 5A, two way ANOVA, STN-DBS x PPX interaction: $F_{1,14} = 5.759$, $p = .031$).
33
34 16 These effects are likely to reflect changes in behavioral activity rather than alteration of
35
36 17 motor skills *per se*, first because neither PPX nor STN-DBS affected the ambulatory speed
37
38 18 during the open area test (Figure 5B, Two way ANOVA, STN-DBS x PPX interaction $F_{1,48} =$
39
40 19 $.214$, $p = .650$) and second, adjustments during the stepping test were not impacted by either
41
42 20 treatment (Figure 5B, two ways ANOVA, STN-DBS x PPX interaction: left to right moves, left
43
44 21 limb: $F_{1,18} = .184$, $p = .673$; right limb: $F_{1,18} = .653$, $p = .983$; right to left moves, left limb: $F_{1,18}$
45
46 22 $= .0017$, $p = .915$; right limb: $F_{1,18} = .764$, $p = .393$).

47
48
49 23 Taken together, these results suggest that STN-DBS exacerbates the loss of
50
51 24 motivation induced by the dopaminergic lesion and reduce behavioral activity, effects that
52
53 25 were alleviated by PPX.
54
55
56 26
57
58 27
59
60

STN-DBS and Apathy in Parkinson's Disease

Discussion

Combining a relevant *in vivo* model of neuropsychiatric PD symptoms, chronic bilateral STN-DBS in awake and freely moving animals and a pharmacological approach, we provide strong evidence for a direct deleterious effect of STN-DBS on motivation. STN-DBS induces a significant deficit in motivated behavior, due neither to a reward sensitivity deficit related to a potential anhedonic effect, nor to motor deficits. This behavioral effect was completely reversed by the activation of D₂R/D₃R with PPX, revealing the potential critical role of DA in this STN-DBS motivational effect.

It is essential to better understand the pathophysiology of neuropsychiatric symptoms of PD and how they respond to current treatments such as STN-DBS. Here, we tried to be as close as possible to the clinical situation in PD, especially for the stimulation conditions. While most preclinical studies have been performed with acute STN-DBS that was applied daily only during the tasks and experiments (e.g.,^{41, 49}), the novel stimulation system we used allowed continuous STN-DBS for several days. In addition, we used monopolar stimulation. Indeed, it is the most widely applied in PD patients¹ as well as in studies describing apathy under STN-DBS^{17, 21, 48, 61-64}. Furthermore, a preclinical study in rat has clearly demonstrated that it reduces tissue damage compared to bipolar stimulation.⁶⁵ Moreover, although monopolar stimulation is more likely to affect the surrounding STN fibers than bipolar stimulation, it was applied at intensities below 225 μ A to minimize current spreading as previously demonstrated.⁶⁶ The motivational dimension of apathy especially concerns hum-drum daily tasks that are neither challenging nor particularly effortful.⁴ A sucrose self-administration procedure with a 2.5 % solution, in a fixed ratio 1 schedule, in non-food-deprived rats, is an appropriate way to operationalize a simple effort with a relatively moderate rewarding outcome. This approach has allowed us to demonstrate a clear effect of STN-DBS on motivated behaviors during an operant task. The decrease of baseline ambulatory activity detected during the open area test, unrelated to motor impairment, further suggests that the STN-DBS-induced operant deficit may be considered as a decrease in the maintenance of

STN-DBS and Apathy in Parkinson's Disease

1 behavioral activity, as observed in apathy.⁹ While we previously provided strong evidence
2 that the dopaminergic denervation can account for the loss of motivation in PD, here we
3 report that STN-DBS by itself could induce or exacerbate this motivational impairment via
4 DA-driven mechanisms, independent of the hypodopaminergy induced by DRT reduction.
5 Furthermore, the present study provides a missing link, reconciling apparently contradictory
6 clinical observations on the origin of post-operative apathy in stimulated PD patients.

7 The myriad of reported neuropsychiatric side effects of STN-DBS in PD patients³ has
8 triggered several animal studies to understand their phenomenology. These studies have
9 demonstrated the possible implication of STN-DBS in PD-related depression^{49, 67} or potent
10 effects on impulsive behaviors,⁶⁸ but few of them tackled the effects on pure motivational
11 processes. As far as we know, only one report mentions data that could be interpreted as a
12 transient reduced motivation under STN-DBS.⁶⁹ However, another study showed no effect of
13 STN-DBS on operant activity in a fixed-ratio schedule of reinforcement and even described
14 an increase in performance in obtaining a sucrose pellet during a progressive ratio
15 schedule.⁴¹ Major differences between this previous study and our protocol are likely to
16 account for these discrepancies. Indeed, Rouaud et al., used acute bipolar stimulation
17 conditions compared to our chronic monopolar STN-DBS; interestingly, psychiatric side
18 effects of STN-DBS strongly depend on electrode polarity. For example, acute depressive
19 states following regular monopolar STN-DBS are greatly reduced by switching to the scarcer
20 bipolar STN-DBS.^{70, 71} Moreover, the design of the operant task was different enough to yield
21 diverging results. In particular, our study avoided food-restriction. Whereas all the studies
22 having inactivated the STN found an increase in motivation for food in restricted animals, one
23 study demonstrated that this effect was completely occluded in rats fed *ad libitum*.³⁷ Finally,
24 while the conditioned place preference revealed an effect of STN-DBS on the rewarding
25 properties of sucrose in this study,⁴¹ we did not find any modification of this parameter during
26 the two bottle choice test.

27 The STN can be functionally and anatomically subdivided into motor, associative and
28 limbic sub-territories.⁷²⁻⁷⁴ Then it is part of the three respective basal ganglia loops.^{73, 74} In PD

STN-DBS and Apathy in Parkinson's Disease

1 patients, DBS is tailored to stimulate the motor part of the STN, and so to impact the whole
2 motor circuitry. However, an inaccurate electrode placement within the STN can engage the
3 circuitries involving its associative or limbic part rather than the motor one,⁷⁵ potentially
4 leading to non-motor side effects,^{70, 74} apathy in particular. In line with this assumption, a very
5 recent study succeeded in alleviating post STN-DBS apathy in patients by displacing the
6 electrode from the limbic STN to the motor part,²³ demonstrating that apathy under STN-DBS
7 could rely on a mechanism caused by the stimulation of the limbic part of the STN. Our data
8 suggest that the dopaminergic system might be involved in this mechanism, at least in part.

9 In patients, apathy occurring after STN-DBS can be treated by dopaminergic
10 agonists, including those targeting the D₂R and D₃R.^{18, 19} This, and the fact that
11 methylphenidate can alleviate fatigue⁷⁶, which is a related and/or confounding symptom of
12 apathy⁷⁷, has prompted the hypothesis that a strong hypodopaminergic state revealed by
13 reduction of DRT, rather than STN-DBS *per se*, may be responsible for the resurgence of
14 apathy.¹³ Using a pharmacological protocol allowing exploration of the chronic effects of PPX
15 and known to increase D₂R and D₃R expression,⁵⁹ we demonstrated that PPX, a D₂R/D₃R
16 agonist, fully rescued the motivational deficit induced by STN-DBS in control and lesioned
17 rats. It should be noted that this pharmacological protocol was not able to reverse the loss of
18 motivation due to the dopaminergic lesion, indicating that even if both the lesion and STN-
19 DBS effects on motivation could be driven by dopaminergic mechanisms, these could be
20 significantly different. This suggests that STN-DBS may impact on behavior via its own
21 effects on DA transmission, which is consistent with previous animal model studies showing
22 that STN-DBS modulates DA system.^{58, 78-82} The three so-called basal ganglia limbic,
23 associative and motor loops including the STN are not completely segregated; the different
24 structures involved are functionally interconnected⁸³ by an ascending striato-midbrain-striatal
25 spiraling circuitry.⁸⁴ Moreover, beyond this complex organization, which has yet to be
26 demonstrated in rats, SNc and VTA are overlapping structures⁸⁵ and the whole striatum is
27 characterized by a dense local microcircuitry^{86, 87} which could also contribute to this
28 interconnection. Thus, STN-DBS may modulate DA transmission within the basal ganglia

STN-DBS and Apathy in Parkinson's Disease

1 and, through these subcortical interactions, disrupt the complex chain of events that
2 transforms intentions into adapted action, and thereby influence non-motor functions.^{6, 9}
3 Nonetheless, we cannot exclude a potential involvement of the structures surrounding the
4 STN . It is unlikely that the electric field emanating from the electrode is restrained to the
5 STN borders and DBS of some of these structures in the vicinity of the STN, such as the
6 lateral hypothalamus, can also modulate DA transmission⁸⁸. We previously found that short
7 STN-DBS can decrease the level of D₂R and D₃R within the NAc.⁵⁸ Given the critical
8 implication of the D₂R and D₃R in motivational processes,⁸⁹⁻⁹² our data suggest a causal
9 involvement of these receptors in the motivational deficit that we observed. In addition, D₂R
10 and D₃R are expressed in several other non-motor structures impacted by PPX treatment⁹³
11 and some of them are part of down-stream circuits with metabolic activities known to be
12 affected by STN-DBS.^{22, 94} Thus, beyond the NAc, both STN-DBS and PPX effects could
13 differentially engage some common system responsible for behavior changes highlighted in
14 this study. However, dopaminergic agonists do not always efficiently alleviate apathy under
15 STN-DBS²². Non dopaminergic lesions frequently occur during the course of disease^{95, 96},
16 and serotonin for example is proposed to participate in PD apathy⁹⁷. In rats, STN-DBS has
17 been shown to decrease serotonin release promoting depressive like behavior.^{49, 98} Thus,
18 we cannot completely exclude the implication of other neurotransmitters in the loss of
19 motivation observed in patients or in this study.

20 As previously reported,⁹⁹ PPX at 0.3 mg/kg induces hyperlocomotricity in the open
21 area test in control rats. This hyperactivity is unlikely to be responsible for the reversion of
22 the self-administration deficit observed in PPX-treated STN-DBS rats since first, the level of
23 non-stimulated treated rats remains similar to that of control rats, and second, PPX induced
24 hypoactivity in non-stimulated lesioned rats whereas it also reversed the operant deficit
25 induced by STN-DBS in those lesioned rats. This hypoactivity promoted by PPX in lesioned
26 rats has previously been reported during the 120 minutes post injection window in control
27 animals⁹⁹ and could rely on a sedating effect mediated by the presynaptic D₃R¹⁰⁰. We chose
28 to separate the behavioral test from injection by 3h to avoid this effect. Since we previously

STN-DBS and Apathy in Parkinson's Disease

1 demonstrated that the 6-OHDA lesion modifies D₃R expression within the dorsolateral part of
2 the striatum¹⁰¹, local imbalance between pre and post synaptic D₂R and/or D₃R expression
3 or activity¹⁰² could be responsible for DA transmission blunting, thus promoting this
4 hypoactivity. Very intriguingly, in some rats of both the control and lesioned groups, the
5 combination of STN-DBS and PPX induced a very high- level of activity that could emphasize
6 the importance of individual traits, as demonstrated for the expression or severity of impulse
7 control disorder in PD¹⁰³⁻¹⁰⁵ or compulsive drug taking.¹⁰⁶ Thus, depending on complex
8 interactions with dopaminergic treatments and according to pre-existing individual traits,
9 STN-DBS could also lead to the development of hyperdopaminergic states reminiscent of
10 hypomanic behaviors also observed in PD patients under STN-DBS.^{62, 74, 107, 108}

11 Altogether, these data bring coherence to clinical observations that seemed
12 contradictory until today: apathy in PD patients, at least its motivational dimension, could be
13 induced by STN-DBS itself and attenuated by activation of D₂R/D₃R, regardless of (or in
14 addition to) DRT reduction and the DA-mediated hypofunction. More detailed molecular
15 analysis or techniques such as optogenetics could enable us to clarify the structures or sub-
16 territories involved in this STN-DBS induced DA-driven loss of motivation. While it is beyond
17 the scope of the present study, it would be also of interest to further investigate the effect of
18 STN-DBS on the emotional and affective component of apathy, as STN has been proposed
19 to be involved in such processes¹⁰⁹.

20 STN-DBS has been shown to prevent cocaine or heroin re-escalation intake in rats⁴⁰,
21⁴⁴ and to reduce dopamine dysregulation syndrome or addictive behavior such as
22 pathological gambling.¹¹⁰⁻¹¹⁴ Thus, STN-DBS is currently explored as an effective treatment
23 for addiction.^{45, 115} However, our results suggest that this apparent beneficial effect could be
24 underlain by a general motivational deficit. In rats, inhibiting the STN can abolish affective
25 responses for salient reward¹⁰⁹ and STN-DBS can induce depression-like behaviors.^{49, 67, 116}
26 Furthermore, PD patients for whom addictive behavior was reduced under STN-DBS can
27 also co-express apathy.^{14, 110} These convergent facts are raising the question of the nature of
28 this apparent "anti-addictive" effect of STN-DBS. This neurosurgical treatment could rather

STN-DBS and Apathy in Parkinson's Disease

1
2
3 1 induce a state of negative affect, comparable to a certain extent to the blunted dopaminergic
4
5 2 transmission observed during prolonged drug consumption¹¹⁷⁻¹¹⁹ , increasing the risk of
6
7 3 addictive behavior resurgence in the same way as drugs of abuse withdrawal syndrome
8
9 4 promotes relapse.¹²⁰
10

11 5 Thus, this new insight calls for reconsideration of the role of STN-DBS on mood in PD
12
13 6 to improve patient care and quality of life, and more broadly, to rethink the use of STN-DBS
14
15 7 in psychiatry.
16
17
18 8
19
20 9
21
22 10
23
24 11
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

STN-DBS and Apathy in Parkinson's Disease

1 Financial disclosure

2 This work was supported by the Institut National de la Santé et de la recherche Médicale,
3 Fondation de France, Région Rhône-Alpes (Arc n°2), Neurodis, Grenoble Alpes Université,
4 CNRS, Aix-Marseille Université and France Parkinson. The authors declare no conflict of
5 interest.

7 Acknowledgments

8 We would like to thank Fiona Hemming for her critical reading, as well as Séverine Lirzin and
9 Olivier Mainard for the maintenance and their technical help concerning the micro-stimulator
10 system.

12 Author contributions

13 YV, SB, SC and MS were responsible for the conception and the design of the study. YV,
14 SB, CC and RM carried out the stereotaxic surgeries and the post-surgery monitoring of the
15 animals. YV, CC and SB performed the neuroanatomical analysis of electrode implantation
16 and lesions. YV and SB carried out the behavioral experiments and analysis. LKLG and PS
17 provided micro-stimulators as well as training and advice for their use. YV and SB wrote the
18 paper with the help of the other authors.

19
20
21 Supplementary information accompanies this paper.

STN-DBS and Apathy in Parkinson's Disease

References

1. Benabid AL, Chabardes S, Mitrofanis J, Pollak P. Deep brain stimulation of the subthalamic nucleus for the treatment of Parkinson's disease. *Lancet Neurol* 2009;8(1):67-81.
2. Chaudhuri KR, Schapira AH. Non-motor symptoms of Parkinson's disease: dopaminergic pathophysiology and treatment. *Lancet Neurol* 2009;8(5):464-474.
3. Castrioto A, Lhommee E, Moro E, Krack P. Mood and behavioural effects of subthalamic stimulation in Parkinson's disease. *Lancet Neurol* 2014;13(3):287-305.
4. Marin RS. Apathy: Concept, Syndrome, Neural Mechanisms, and Treatment. *Semin Clin Neuropsychiatry* 1996;1(4):304-314.
5. Martinez-Fernandez R, Pelissier P, Quesada JL, et al. Postoperative apathy can neutralise benefits in quality of life after subthalamic stimulation for Parkinson's disease. *Journal of neurology, neurosurgery, and psychiatry* 2016;87(3):311-318.
6. Levy R, Dubois B. Apathy and the functional anatomy of the prefrontal cortex-basal ganglia circuits. *Cereb Cortex* 2006;16(7):916-928.
7. Chagraoui A, Boukharz L, Thibaut F, Anouar Y, Maltete D. The pathophysiological mechanisms of motivational deficits in Parkinson's disease. *Progress in neuro-psychopharmacology & biological psychiatry* 2018;81:138-152.
8. Chaudhuri KR, Odin P. The challenge of non-motor symptoms in Parkinson's disease. *Prog Brain Res* 2010;184:325-341.
9. Pagonabarraga J, Kulisevsky J, Strafella AP, Krack P. Apathy in Parkinson's disease: clinical features, neural substrates, diagnosis, and treatment. *Lancet Neurol* 2015;14(5):518-531.
10. Sierra M, Carnicella S, Strafella AP, et al. Apathy and Impulse Control Disorders: Yin & Yang of Dopamine Dependent Behaviors. *Journal of Parkinson's disease* 2015;5(3):625-636.
11. Maier F, Lewis CJ, Horstkoetter N, et al. Subjective perceived outcome of subthalamic deep brain stimulation in Parkinson's disease one year after surgery. *Parkinsonism & related disorders* 2016;24:41-47.
12. Maier F, Lewis CJ, Horstkoetter N, et al. Patients' expectations of deep brain stimulation, and subjective perceived outcome related to clinical measures in Parkinson's disease: a mixed-method approach. *Journal of neurology, neurosurgery, and psychiatry* 2013;84(11):1273-1281.
13. Aarsland D, Bronnick K, Alves G, et al. The spectrum of neuropsychiatric symptoms in patients with early untreated Parkinson's disease. *Journal of neurology, neurosurgery, and psychiatry* 2009;80(8):928-930.
14. Abbes M, Lhommee E, Thobois S, et al. Subthalamic stimulation and neuropsychiatric symptoms in Parkinson's disease: results from a long-term follow-up cohort study. *Journal of neurology, neurosurgery, and psychiatry* 2018;89(8):836-843.
15. Drapier D, Peron J, Leray E, et al. Emotion recognition impairment and apathy after subthalamic nucleus stimulation in Parkinson's disease have separate neural substrates. *Neuropsychologia* 2008;46(11):2796-2801.
16. Witt K, Daniels C, Reiff J, et al. Neuropsychological and psychiatric changes after deep brain stimulation for Parkinson's disease: a randomised, multicentre study. *Lancet Neurol* 2008;7(7):605-614.
17. Thobois S, Ardouin C, Lhommee E, et al. Non-motor dopamine withdrawal syndrome after surgery for Parkinson's disease: predictors and underlying mesolimbic denervation. *Brain : a journal of neurology* 2010;133(Pt 4):1111-1127.

STN-DBS and Apathy in Parkinson's Disease

18. Thobois S, Lhomme E, Klinger H, et al. Parkinsonian apathy responds to dopaminergic stimulation of D2/D3 receptors with piribedil. *Brain : a journal of neurology* 2013;136(Pt 5):1568-1577.
19. Czernecki V, Schupbach M, Yaici S, et al. Apathy following subthalamic stimulation in Parkinson disease: a dopamine responsive symptom. *Movement disorders : official journal of the Movement Disorder Society* 2008;23(7):964-969.
20. Magnard R, Vachez Y, Carcenac C, et al. What can rodent models tell us about apathy and associated neuropsychiatric symptoms in Parkinson's disease? *Transl Psychiatry* 2016;6:e753.
21. Drapier D, Drapier S, Sauleau P, et al. Does subthalamic nucleus stimulation induce apathy in Parkinson's disease? *Journal of neurology* 2006;253(8):1083-1091.
22. Le Jeune F, Drapier D, Bourguignon A, et al. Subthalamic nucleus stimulation in Parkinson disease induces apathy: a PET study. *Neurology* 2009;73(21):1746-1751.
23. Zoon TJ, de Bie RM, Schuurman PR, van den Munckhof P, Denys D, Figeet M. Resolution of apathy after dorsal instead of ventral subthalamic deep brain stimulation for Parkinson's disease. *Journal of neurology* 2019.
24. Breyse E, Pelloux Y, Baunez C. The Good and Bad Differentially Encoded within the Subthalamic Nucleus in Rats(1,2,3). *eNeuro* 2015;2(5).
25. Darbaky Y, Baunez C, Arecchi P, Legallet E, Apicella P. Reward-related neuronal activity in the subthalamic nucleus of the monkey. *Neuroreport* 2005;16(11):1241-1244.
26. Espinosa-Parrilla JF, Baunez C, Apicella P. Modulation of neuronal activity by reward identity in the monkey subthalamic nucleus. *The European journal of neuroscience* 2015;42(1):1705-1717.
27. Lardeux S, Pernaud R, Paleressompoulle D, Baunez C. Beyond the reward pathway: coding reward magnitude and error in the rat subthalamic nucleus. *J Neurophysiol* 2009;102(4):2526-2537.
28. Montanari C, Giorla E, Pelloux Y, Baunez C. Subthalamic nucleus mediates the modulation on cocaine self-administration induced by ultrasonic vocalization playback in rats. *Addiction biology* 2018.
29. Lardeux S, Paleressompoulle D, Pernaud R, Cador M, Baunez C. Different populations of subthalamic neurons encode cocaine vs. sucrose reward and predict future error. *J Neurophysiol* 2013;110(7):1497-1510.
30. Justin Rossi P, Peden C, Castellanos O, Foote KD, Gunduz A, Okun MS. The human subthalamic nucleus and globus pallidus internus differentially encode reward during action control. *Human brain mapping* 2017;38(4):1952-1964.
31. Zenon A, Duclos Y, Carron R, et al. The human subthalamic nucleus encodes the subjective value of reward and the cost of effort during decision-making. *Brain : a journal of neurology* 2016;139(Pt 6):1830-1843.
32. Baunez C, Amalric M, Robbins TW. Enhanced food-related motivation after bilateral lesions of the subthalamic nucleus. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2002;22(2):562-568.
33. Baunez C, Dias C, Cador M, Amalric M. The subthalamic nucleus exerts opposite control on cocaine and 'natural' rewards. *Nat Neurosci* 2005;8(4):484-489.
34. Bezzina G, Boon FS, Hampson CL, et al. Effect of quinolinic acid-induced lesions of the subthalamic nucleus on performance on a progressive-ratio schedule of reinforcement: a quantitative analysis. *Behavioural brain research* 2008;195(2):223-230.
35. Uslaner JM, Dell'Orco JM, Pevzner A, Robinson TE. The influence of subthalamic nucleus lesions on sign-tracking to stimuli paired with food and drug rewards: facilitation of incentive salience attribution? *Neuropsychopharmacology* 2008;33(10):2352-2361.

STN-DBS and Apathy in Parkinson's Disease

- 1
2
3 1 36. Maillard CA, Edwards DA. [Subthalamic lesions eliminate sexual behavior in the
4 2 male rat]. *Journal de physiologie* 1988;83(1):50-55.
- 5 3 37. Pratt WE, Choi E, Guy EG. An examination of the effects of subthalamic nucleus
6 4 inhibition or mu-opioid receptor stimulation on food-directed motivation in the non-deprived
7 5 rat. *Behavioural brain research* 2012;230(2):365-373.
- 8 6 38. Eusebio A, Witjas T, Cohen J, et al. Subthalamic nucleus stimulation and compulsive
9 7 use of dopaminergic medication in Parkinson's disease. *Journal of neurology, neurosurgery,*
10 8 *and psychiatry* 2013;84(8):868-874.
- 11 9 39. Lhomme E, Klinger H, Thobois S, et al. Subthalamic stimulation in Parkinson's
12 10 disease: restoring the balance of motivated behaviours. *Brain : a journal of neurology*
13 11 2012;135(Pt 5):1463-1477.
- 14 12 40. Pelloux Y, Degoulet M, Tiran-Cappello A, et al. Subthalamic nucleus high frequency
15 13 stimulation prevents and reverses escalated cocaine use. *Molecular psychiatry*
16 14 2018;23(12):2266-2276.
- 17 15 41. Rouaud T, Lardeux S, Panayotis N, Paleressompoulle D, Cador M, Baunez C.
18 16 Reducing the desire for cocaine with subthalamic nucleus deep brain stimulation. *Proceedings*
19 17 *of the National Academy of Sciences of the United States of America* 2010;107(3):1196-
20 18 1200.
- 21 19 42. Adams WK, Vonder Haar C, Tremblay M, et al. Deep-Brain Stimulation of the
22 20 Subthalamic Nucleus Selectively Decreases Risky Choice in Risk-Preferring Rats. *eNeuro*
23 21 2017;4(4).
- 24 22 43. Hachem-Delaunay S, Fournier ML, Cohen C, et al. Subthalamic nucleus high-
25 23 frequency stimulation modulates neuronal reactivity to cocaine within the reward circuit.
26 24 *Neurobiol Dis* 2015;80:54-62.
- 27 25 44. Wade CL, Kallupi M, Hernandez DO, et al. High-Frequency Stimulation of the
28 26 Subthalamic Nucleus Blocks Compulsive-Like Re-Escalation of Heroin Taking in Rats.
29 27 *Neuropsychopharmacology* 2017;42(9):1850-1859.
- 30 28 45. Creed M. Current and emerging neuromodulation therapies for addiction: insight from
31 29 pre-clinical studies. *Curr Opin Neurobiol* 2018;49:168-174.
- 32 30 46. Barone P, Antonini A, Colosimo C, et al. The PRIAMO study: A multicenter
33 31 assessment of nonmotor symptoms and their impact on quality of life in Parkinson's disease.
34 32 *Movement disorders : official journal of the Movement Disorder Society* 2009;24(11):1641-
35 33 1649.
- 36 34 47. Pagonabarraga J, Kulisevsky J. Apathy in Parkinson's Disease. *International review of*
37 35 *neurobiology* 2017;133:657-678.
- 38 36 48. Castelli L, Perozzo P, Zibetti M, et al. Chronic deep brain stimulation of the
39 37 subthalamic nucleus for Parkinson's disease: effects on cognition, mood, anxiety and
40 38 personality traits. *European neurology* 2006;55(3):136-144.
- 41 39 49. Temel Y, Boothman LJ, Blokland A, et al. Inhibition of 5-HT neuron activity and
42 40 induction of depressive-like behavior by high-frequency stimulation of the subthalamic
43 41 nucleus. *Proc Natl Acad Sci U S A* 2007;104(43):17087-17092.
- 44 42 50. Temel Y, Tan S, Vlamings R, et al. Cognitive and limbic effects of deep brain
45 43 stimulation in preclinical studies. *Front Biosci* 2009;14:1891-1901.
- 46 44 51. Czernecki V, Pillon B, Houeto JL, et al. Does bilateral stimulation of the subthalamic
47 45 nucleus aggravate apathy in Parkinson's disease? *Journal of neurology, neurosurgery, and*
48 46 *psychiatry* 2005;76(6):775-779.
- 49 47 52. Robert GH, Le Jeune F, Lozachmeur C, et al. Preoperative factors of apathy in
50 48 subthalamic stimulated Parkinson disease: a PET study. *Neurology* 2014;83(18):1620-1626.
- 51
52
53
54
55
56
57
58
59
60

STN-DBS and Apathy in Parkinson's Disease

- 1
2
3 1 53. Wang Y, Li Y, Zhang X, Xie A. Apathy following Bilateral Deep Brain Stimulation of
4 2 Subthalamic Nucleus in Parkinson's Disease: A Meta-Analysis. *Parkinsons Dis*
5 3 2018;2018:9756468.
6 4 54. Forni C, Mainard O, Melon C, Goguenheim D, Kerkerian-Le Goff L, Salin P. Portable
7 5 microstimulator for chronic deep brain stimulation in freely moving rats. *Journal of*
8 6 *neuroscience methods* 2012;209(1):50-57.
9 7 55. Drui G, Carnicella S, Carcenac C, et al. Loss of dopaminergic nigrostriatal neurons
10 8 accounts for the motivational and affective deficits in Parkinson's disease. *Mol Psychiatry*
11 9 2014;19(3):358-367.
12 10 56. Leentjens AF, Koester J, Fruh B, Shephard DT, Barone P, Houben JJ. The effect of
13 11 pramipexole on mood and motivational symptoms in Parkinson's disease: a meta-analysis of
14 12 placebo-controlled studies. *Clin Ther* 2009;31(1):89-98.
15 13 57. Favier M, Duran T, Carcenac C, Drui G, Savasta M, Carnicella S. Pramipexole
16 14 reverses Parkinson's disease-related motivational deficits in rats. *Movement disorders :*
17 15 *official journal of the Movement Disorder Society* 2014;29(7):912-920.
18 16 58. Carcenac C, Favier M, Vachez Y, et al. Subthalamic deep brain stimulation differently
19 17 alters striatal dopaminergic receptor levels in rats. *Movement disorders : official journal of the*
20 18 *Movement Disorder Society* 2015;30(13):1739-1749.
21 19 59. Tokunaga N, Choudhury ME, Nishikawa N, et al. Pramipexole upregulates dopamine
22 20 receptor D(2) and D(3) expression in rat striatum. *J Pharmacol Sci* 2012;120(2):133-137.
23 21 60. Micallef J, Rey M, Eusebio A, et al. Antiparkinsonian drug-induced sleepiness: a
24 22 double-blind placebo-controlled study of L-dopa, bromocriptine and pramipexole in healthy
25 23 subjects. *Br J Clin Pharmacol* 2009;67(3):333-340.
26 24 61. Castelli L, Lanotte M, Zibetti M, et al. Apathy and verbal fluency in STN-stimulated
27 25 PD patients. An observational follow-up study. *Journal of neurology* 2007;254(9):1238-1243.
28 26 62. Funkiewiez A, Ardouin C, Caputo E, et al. Long term effects of bilateral subthalamic
29 27 nucleus stimulation on cognitive function, mood, and behaviour in Parkinson's disease.
30 28 *Journal of neurology, neurosurgery, and psychiatry* 2004;75(6):834-839.
31 29 63. Gervais-Bernard H, Xie-Brustolin J, Mertens P, et al. Bilateral subthalamic nucleus
32 30 stimulation in advanced Parkinson's disease: five year follow-up. *Journal of neurology*
33 31 2009;256(2):225-233.
34 32 64. Schupbach WM, Chastan N, Welter ML, et al. Stimulation of the subthalamic nucleus
35 33 in Parkinson's disease: a 5 year follow up. *Journal of neurology, neurosurgery, and psychiatry*
36 34 2005;76(12):1640-1644.
37 35 65. Temel Y, Visser-Vandewalle V, van der Wolf M, et al. Monopolar versus bipolar high
38 36 frequency stimulation in the rat subthalamic nucleus: differences in histological damage.
39 37 *Neuroscience letters* 2004;367(1):92-96.
40 38 66. Chaturvedi A, Lujan JL, McIntyre CC. Artificial neural network based
41 39 characterization of the volume of tissue activated during deep brain stimulation. *Journal of*
42 40 *neural engineering* 2013;10(5):056023.
43 41 67. Creed MC, Hamani C, Nobrega JN. Effects of repeated deep brain stimulation on
44 42 depressive- and anxiety-like behavior in rats: comparing entopeduncular and subthalamic
45 43 nuclei. *Brain Stimul* 2013;6(4):506-514.
46 44 68. Aleksandrova LR, Creed MC, Fletcher PJ, Lobo DS, Hamani C, Nobrega JN. Deep
47 45 brain stimulation of the subthalamic nucleus increases premature responding in a rat gambling
48 46 task. *Behavioural brain research* 2013;245:76-82.
49 47 69. Darbaky Y, Forni C, Amalric M, Baunez C. High frequency stimulation of the
50 48 subthalamic nucleus has beneficial antiparkinsonian effects on motor functions in rats, but
51 49 less efficiency in a choice reaction time task. *Eur J Neurosci* 2003;18(4):951-956.
52
53
54
55
56
57
58
59
60

STN-DBS and Apathy in Parkinson's Disease

- 1
2
3 1 70. Abulseoud OA, Kasasbeh A, Min HK, et al. Stimulation-Induced Transient Nonmotor
4 2 Psychiatric Symptoms following Subthalamic Deep Brain Stimulation in Patients with
5 3 Parkinson's Disease: Association with Clinical Outcomes and Neuroanatomical Correlates.
6 4 *Stereotactic and functional neurosurgery* 2016;94(2):93-101.
- 7 5 71. Simmons DB, Dashtipour K. The Quantitative Measurement of Reversible Acute
8 6 Depression after Subthalamic Deep Brain Stimulation in a Patient with Parkinson Disease.
9 7 *Case reports in neurological medicine* 2015;2015:165846.
- 10 8 72. Alkemade A, Schnitzler A, Forstmann BU. Topographic organization of the human
11 9 and non-human primate subthalamic nucleus. *Brain Struct Funct* 2015;220(6):3075-3086.
- 12 10 73. Lambert C, Zrinzo L, Nagy Z, et al. Confirmation of functional zones within the
13 11 human subthalamic nucleus: patterns of connectivity and sub-parcellation using diffusion
14 12 weighted imaging. *NeuroImage* 2012;60(1):83-94.
- 15 13 74. Mallet L, Schupbach M, N'Diaye K, et al. Stimulation of subterritories of the
16 14 subthalamic nucleus reveals its role in the integration of the emotional and motor aspects of
17 15 behavior. *Proc Natl Acad Sci U S A* 2007;104(25):10661-10666.
- 18 16 75. Stefurak T, Mikulis D, Mayberg H, et al. Deep brain stimulation for Parkinson's
19 17 disease dissociates mood and motor circuits: a functional MRI case study. *Movement*
20 18 *disorders : official journal of the Movement Disorder Society* 2003;18(12):1508-1516.
- 21 19 76. Roji R, Centeno C. The use of methylphenidate to relieve fatigue. *Current opinion in*
22 20 *supportive and palliative care* 2017;11(4):299-305.
- 23 21 77. Skorvanek M, Gdovinova Z, Rosenberger J, et al. The associations between fatigue,
24 22 apathy, and depression in Parkinson's disease. *Acta Neurol Scand* 2015;131(2):80-87.
- 25 23 78. Benazzouz A, Gao D, Ni Z, Benabid AL. High frequency stimulation of the STN
26 24 influences the activity of dopamine neurons in the rat. *Neuroreport* 2000;11(7):1593-1596.
- 27 25 79. Min HK, Ross EK, Jo HJ, et al. Dopamine Release in the Nonhuman Primate Caudate
28 26 and Putamen Depends upon Site of Stimulation in the Subthalamic Nucleus. *The Journal of*
29 27 *neuroscience : the official journal of the Society for Neuroscience* 2016;36(22):6022-6029.
- 30 28 80. Bruet N, Windels F, Bertrand A, Feuerstein C, Poupard A, Savasta M. High frequency
31 29 stimulation of the subthalamic nucleus increases the extracellular contents of striatal
32 30 dopamine in normal and partially dopaminergic denervated rats. *J Neuropathol Exp Neurol*
33 31 2001;60(1):15-24.
- 34 32 81. Kocobicak E, Jahanshahi A, Schonfeld L, Heschem SA, Temel Y, Tan S. Deep Brain
35 33 Stimulation of the Rat Subthalamic Nucleus Induced Inhibition of Median Raphe
36 34 Serotonergic and Dopaminergic Neurotransmission. *Turk Neurosurg* 2015;25(5):721-727.
- 37 35 82. Zhao XD, Cao YQ, Liu HH, Li FQ, You BM, Zhou XP. Long term high frequency
38 36 stimulation of STN increases dopamine in the corpus striatum of hemiparkinsonian rhesus
39 37 monkey. *Brain Res* 2009;1286:230-238.
- 40 38 83. Belin D, Everitt BJ. Cocaine seeking habits depend upon dopamine-dependent serial
41 39 connectivity linking the ventral with the dorsal striatum. *Neuron* 2008;57(3):432-441.
- 42 40 84. Haber SN, Knutson B. The reward circuit: linking primate anatomy and human
43 41 imaging. *Neuropsychopharmacology : official publication of the American College of*
44 42 *Neuropsychopharmacology* 2010;35(1):4-26.
- 45 43 85. Bjorklund A, Dunnett SB. Dopamine neuron systems in the brain: an update. *Trends*
46 44 *in neurosciences* 2007;30(5):194-202.
- 47 45 86. Gittis AH, Kreitzer AC. Striatal microcircuitry and movement disorders. *Trends in*
48 46 *neurosciences* 2012;35(9):557-564.
- 49 47 87. Sesack SR, Grace AA. Cortico-Basal Ganglia reward network: microcircuitry.
50 48 *Neuropsychopharmacology : official publication of the American College of*
51 49 *Neuropsychopharmacology* 2010;35(1):27-47.
- 52
53
54
55
56
57
58
59
60

STN-DBS and Apathy in Parkinson's Disease

- 1 88. Simon MJ, Higuera-Matas A, Roura-Martinez D, et al. Changes in D1 but not D2
2 dopamine or mu-opioid receptor expression in limbic and motor structures after lateral
3 hypothalamus electrical self-stimulation: A quantitative autoradiographic study. *Neurobiology
4 of learning and memory* 2016;127:17-26.
- 5 89. Le Foll B, Goldberg SR, Sokoloff P. The dopamine D3 receptor and drug dependence:
6 effects on reward or beyond? *Neuropharmacology* 2005;49(4):525-541.
- 7 90. Simpson EH, Winiger V, Biezonski DK, Haq I, Kandel ER, Kellendonk C. Selective
8 overexpression of dopamine D3 receptors in the striatum disrupts motivation but not
9 cognition. *Biol Psychiatry* 2014;76(10):823-831.
- 10 91. Drew MR, Simpson EH, Kellendonk C, et al. Transient overexpression of striatal D2
11 receptors impairs operant motivation and interval timing. *The Journal of neuroscience : the
12 official journal of the Society for Neuroscience* 2007;27(29):7731-7739.
- 13 92. Trifilieff P, Feng B, Urizar E, et al. Increasing dopamine D2 receptor expression in the
14 adult nucleus accumbens enhances motivation. *Mol Psychiatry* 2013;18(9):1025-1033.
- 15 93. Ishibashi K, Ishii K, Oda K, Mizusawa H, Ishiwata K. Binding of pramipexole to
16 extrastriatal dopamine D2/D3 receptors in the human brain: a positron emission tomography
17 study using ¹¹C-FLB 457. *PloS one* 2011;6(3):e17723.
- 18 94. Knight EJ, Testini P, Min HK, et al. Motor and Nonmotor Circuitry Activation
19 Induced by Subthalamic Nucleus Deep Brain Stimulation in Patients With Parkinson Disease:
20 Intraoperative Functional Magnetic Resonance Imaging for Deep Brain Stimulation. *Mayo
21 Clinic proceedings* 2015;90(6):773-785.
- 22 95. Carriere N, Besson P, Dujardin K, et al. Apathy in Parkinson's disease is associated
23 with nucleus accumbens atrophy: a magnetic resonance imaging shape analysis. *Movement
24 disorders : official journal of the Movement Disorder Society* 2014;29(7):897-903.
- 25 96. Reijnders JS, Scholtissen B, Weber WE, Aalten P, Verhey FR, Leentjens AF.
26 Neuroanatomical correlates of apathy in Parkinson's disease: A magnetic resonance imaging
27 study using voxel-based morphometry. *Movement disorders : official journal of the
28 Movement Disorder Society* 2010;25(14):2318-2325.
- 29 97. Maillet A, Krack P, Lhommee E, et al. The prominent role of serotonergic
30 degeneration in apathy, anxiety and depression in de novo Parkinson's disease. *Brain : a
31 journal of neurology* 2016;139(Pt 9):2486-2502.
- 32 98. Creed MC, Hamani C, Bridgman A, Fletcher PJ, Nobrega JN. Contribution of
33 decreased serotonin release to the antidyskinetic effects of deep brain stimulation in a rodent
34 model of tardive dyskinesia: comparison of the subthalamic and entopeduncular nuclei. *The
35 Journal of neuroscience : the official journal of the Society for Neuroscience*
36 2012;32(28):9574-9581.
- 37 99. Chang WL, Breier MR, Yang A, Swerdlow NR. Disparate effects of pramipexole on
38 locomotor activity and sensorimotor gating in Sprague-Dawley rats. *Pharmacol Biochem
39 Behav* 2011;99(4):634-638.
- 40 100. Riddle JL, Rokosik SL, Napier TC. Pramipexole- and methamphetamine-induced
41 reward-mediated behavior in a rodent model of Parkinson's disease and controls. *Behavioural
42 brain research* 2012;233(1):15-23.
- 43 101. Favier M, Carcenac C, Drui G, et al. Implication of dorsostriatal D3 receptors in
44 motivational processes: a potential target for neuropsychiatric symptoms in Parkinson's
45 disease. *Sci Rep* 2017;7:41589.
- 46 102. Hurley MJ, Jenner P. What has been learnt from study of dopamine receptors in
47 Parkinson's disease? *Pharmacol Ther* 2006;111(3):715-728.
- 48 103. Marin-Lahoz J, Pagonabarraga J, Martinez-Horta S, et al. Parkinson's Disease:
49 Impulsivity Does Not Cause Impulse Control Disorders but Boosts Their Severity. *Frontiers
50 in psychiatry* 2018;9:465.

STN-DBS and Apathy in Parkinson's Disease

- 1
2
3 1 104. Weintraub D, Koester J, Potenza MN, et al. Impulse control disorders in Parkinson
4 2 disease: a cross-sectional study of 3090 patients. *Arch Neurol* 2010;67(5):589-595.
5 3 105. Houeto JL, Magnard R, Dalley JW, Belin D, Carnicella S. Trait Impulsivity and
6 4 Anhedonia: Two Gateways for the Development of Impulse Control Disorders in Parkinson's
7 5 Disease? *Front Psychiatry* 2016;7:91.
8 6 106. Belin D, Mar AC, Dalley JW, Robbins TW, Everitt BJ. High impulsivity predicts the
9 7 switch to compulsive cocaine-taking. *Science (New York, NY)* 2008;320(5881):1352-1355.
10 8 107. Scangos KW, Shahlaie K. Acute Frequency-Dependent Hypomania Induced by
11 9 Ventral Subthalamic Nucleus Deep Brain Stimulation in Parkinson's Disease: A Case Report.
12 10 *Biological psychiatry* 2017;82(5):e39-e41.
13 11 108. Welter ML, Schupbach M, Czernecki V, et al. Optimal target localization for
14 12 subthalamic stimulation in patients with Parkinson disease. *Neurology* 2014;82(15):1352-
15 13 1361.
16 14 109. Pelloux Y, Meffre J, Giorla E, Baunez C. The subthalamic nucleus keeps you high on
17 15 emotion: behavioral consequences of its inactivation. *Frontiers in behavioral neuroscience*
18 16 2014;8:414.
19 17 110. Ardouin C, Voon V, Worbe Y, et al. Pathological gambling in Parkinson's disease
20 18 improves on chronic subthalamic nucleus stimulation. *Movement disorders : official journal*
21 19 *of the Movement Disorder Society* 2006;21(11):1941-1946.
22 20 111. Bandini F, Primavera A, Pizzorno M, Cocito L. Using STN DBS and medication
23 21 reduction as a strategy to treat pathological gambling in Parkinson's disease. *Parkinsonism &*
24 22 *related disorders* 2007;13(6):369-371.
25 23 112. Knobel D, Aybek S, Pollo C, Vingerhoets FJ, Berney A. Rapid resolution of dopamine
26 24 dysregulation syndrome (DDS) after subthalamic DBS for Parkinson disease (PD): a case
27 25 report. *Cognitive and behavioral neurology : official journal of the Society for Behavioral and*
28 26 *Cognitive Neurology* 2008;21(3):187-189.
29 27 113. Witjas T, Baunez C, Henry JM, et al. Addiction in Parkinson's disease: impact of
30 28 subthalamic nucleus deep brain stimulation. *Movement disorders : official journal of the*
31 29 *Movement Disorder Society* 2005;20(8):1052-1055.
32 30 114. Castrioto A, Carnicella S, Fraix V, Chabardes S, Moro E, Krack P. Reversing
33 31 dopaminergic sensitization. *Movement disorders : official journal of the Movement Disorder*
34 32 *Society* 2017;32(12):1679-1683.
35 33 115. Pelloux Y, Baunez C. Deep brain stimulation for addiction: why the subthalamic
36 34 nucleus should be favored. *Curr Opin Neurobiol* 2013;23(4):713-720.
37 35 116. Tan SK, Janssen ML, Jahanshahi A, et al. High frequency stimulation of the
38 36 subthalamic nucleus increases c-fos immunoreactivity in the dorsal raphe nucleus and afferent
39 37 brain regions. *J Psychiatr Res* 2011;45(10):1307-1315.
40 38 117. Martinez D, Gil R, Slifstein M, et al. Alcohol dependence is associated with blunted
41 39 dopamine transmission in the ventral striatum. *Biological psychiatry* 2005;58(10):779-786.
42 40 118. Barak S, Carnicella S, Yowell QV, Ron D. Glial cell line-derived neurotrophic factor
43 41 reverses alcohol-induced allostasis of the mesolimbic dopaminergic system: implications for
44 42 alcohol reward and seeking. *The Journal of neuroscience : the official journal of the Society*
45 43 *for Neuroscience* 2011;31(27):9885-9894.
46 44 119. Martinez D, Greene K, Broft A, et al. Lower level of endogenous dopamine in patients
47 45 with cocaine dependence: findings from PET imaging of D(2)/D(3) receptors following acute
48 46 dopamine depletion. *The American journal of psychiatry* 2009;166(10):1170-1177.
49 47 120. Le Moal M, Koob GF. Drug addiction: pathways to the disease and
50 48 pathophysiological perspectives. *Eur Neuropsychopharmacol* 2007;17(6-7):377-393.
51 49 121. Paxinos G WC. *The rat brain in stereotaxic coordinates*. 1998.
52 50
53 50
54
55
56
57
58
59
60

STN-DBS and Apathy in Parkinson's Disease

1
2
3 1
4 2
5 3
6 4
7 4
8 5 **Figures legends**
9 6

10 7 **FIGURE 1:** Experimental design and electrode implantation.

11 8 **(A)** Time course of the study, groups of animals and histological controls

12 9 After learning the sucrose self-administration task, lesion and implantation of electrodes, rats
13 10 were divided into a stimulated and a non-stimulated group according to their performances,
14 11 to constitute initially equal performance groups. Then, after 4 days of subthalamic nucleus
15 12 deep brain stimulation (STN-DBS) with further sucrose self-administration, pramipexole
16 13 (PPX) or vehicle (Veh) was injected twice a day into half of the stimulated (Non lesioned rats:
17 14 STN-DBS + PPX, n = 8; STN-DBS + Veh, n = 13; lesioned rats: Lesion + STN-DBS + PPX, n
18 15 = 5; Lesion + STN-DBS + Veh, n = 5) and non-stimulated rats (Control + PPX, n = 22;
19 16 Control + Veh, n = 22; Lesioned rats: Lesion + PPX, n = 4; Lesion + Veh, n = 6) for 20 days.
20 17 During this time, sucrose self-administration was continued for several days and then
21 18 followed successively by other behavioral tests: the two-bottle choice test, the stepping test
22 19 and finally the open area test. **(B)** Positions of bilateral DBS electrode tips in left and right
23 20 STNs of stimulated animals in representative coronal sections (anteroposterior relative to
24 21 Bregma: -3.60 mm; -3.80 mm; -4.16 mm), reproduced from Paxinos and Watson.¹²¹ Each bar
25 22 represents the placement of one electrode. **(C)** and **(D)** Example of electrode trace within the
26 23 left STN stimulated at 100 μ A **(C)** or 225 μ A **(D)**, after Cresyl violet staining. Scale bar: 1 mm.
27 24 IC, Internal Capsule; LH, Lateral Hypothalamus; ZI, Zona Incerta.

28 25 **(E)** and **(F)** Representative photomicrographs of coronal sections stained for tyrosine
29 26 hydroxylase (TH) in striatal (bottom, 1.6 mm to bregma) and mesencephalic (top, -5.6 mm to
30 27 bregma) regions from naïve (top) and lesioned (bottom) rats.¹²¹ Scale bar = 1 mm. **(G, H)**
31 28 Quantification of TH ImmunoReactivity (IR) loss in the midbrain **(G)** and the striatum **(H)**,
32 29 expressed as the percentage difference compared to the mean value obtained for sham
33 30 operated animals. The SNc bilateral lesion produced an important but partial denervation of

STN-DBS and Apathy in Parkinson's Disease

1 the dorsal striatum. The ventral tegmental area and the nucleus accumbens were preserved
 2 from the lesion. Control + Veh: n = 22; Lesion: n = 20. Control + Veh versus Lesion: * $p < .05$;
 3 *** $p < .001$. DS: dorsal striatum; NAc: nucleus accumbens; SNc: substantia nigra compacta,
 4 VTA: ventral tegmental area.

5
 6 **FIGURE 2:** Subthalamic nucleus deep brain stimulation (STN-DBS) induces a deficit of
 7 preparatory behaviors in 2.5% sucrose self-administration that is rescued by pramipexole
 8 (PPX). **(A)** Time course of the sucrose self-administration experiment. STN-DBS induced a
 9 significant decrease of the number of sucrose deliveries. PPX induced a total reversion of
 10 this deficit. **(B)** Mean sucrose deliveries over the last 3 days of experiment. **(C)** STN-DBS
 11 and PPX did not alter the preference for sucrose.
 12 Control + Veh: n = 22; Control + PPX: n = 22; STN-DBS + Veh: n = 13; STN-DBS + PPX: n =
 13 8. Data shown as means \pm SEM. Control + Veh versus STN-DBS + Veh: * $p < .05$; ** $p < .01$;
 14 STN-DBS + Veh versus STN-DBS + PPX: # $p < .05$; ## $p < .01$.

15
 16 **FIGURE 3:** Subthalamic nucleus deep brain stimulation (STN-DBS) and pramipexole (PPX)
 17 promote hypo and hyper locomotor activity respectively but do not impair motor abilities. **(A)**
 18 Total distance travelled during a 1h open area test. STN-DBS induced a non-significant
 19 tendency towards decreased basal locomotor activity that was counteracted by treatment
 20 with PPX. In control rats, PPX induced a dramatic increase in the distance traveled. **(B)** STN-
 21 DBS and PPX did not change the mean ambulatory speed during the last 2 periods of the
 22 open area test. **(C)** STN-DBS and/or PPX did not alter the fine motor skills of front limbs as
 23 demonstrated by adjusting steps in the course of the stepping test. Control + Veh: n = 22;
 24 Control + PPX: n = 22; STN-DBS + Veh: n = 6; STN-DBS + PPX: n = 8. Data shown as
 25 means \pm SEM. Control + PPX versus Control + Veh: * $p < .05$.

STN-DBS and Apathy in Parkinson's Disease

1
2
3 1 **FIGURE 4:** STN-DBS exacerbates the deficit of preparatory behaviors of the existing
4 motivational deficit in a rodent model of the neuropsychiatric symptoms of PD. **(A)** Time
5 2 course of the sucrose self-administration experiment. STN-DBS decreased the number of
6 3 sucrose deliveries. PPX induced a complete reversion of this deficit. **(B)** Mean sucrose
7 4 deliveries over the last 5 days of experiment. **(C)** STN-DBS and PPX did not alter the
8 5 preference for sucrose in 6-OHDA rats. Lesion + Veh: n = 6; Lesion + PPX: n = 4; Lesion +
9 6 STN-DBS + Veh: n = 5; Lesion + STN-DBS + PPX: n = 5. Data shown as means \pm SEM.
10 7
11 8 Lesion + Veh versus Lesion + STN-DBS + Veh: $*p < .05$; Lesion + STN-DBS + Veh versus
12 9 Lesion + STN-DBS + PPX: $\#p < .05$.

10
11 **FIGURE 5:** Subthalamic nucleus deep brain stimulation (STN-DBS) or pramipexole (PPX)
12 11 individually promote hypoactivity but do not impair motor abilities. **(A)** Total distance traveled
13 12 during a 1h open area test. STN-DBS induced a non-significant tendency towards decreased
14 13 basal locomotor activity. In non-stimulated rats, PPX induced a dramatic decrease in the
15 14 distance traveled. The combination of both treatments increased activity. **(B)** STN-DBS and
16 15 PPX did not change the mean ambulatory speed during the open area test. **(C)** STN-DBS
17 16 and/or PPX did not alter the fine motor skills of front limbs as demonstrated by adjusting
18 17 steps in the course of the stepping test. Lesion + Veh: n = 6; Lesion + PPX: n = 4; Lesion +
19 18 STN-DBS + Veh: n = 5; Lesion + STN-DBS + PPX: n = 5. Data shown as means \pm SEM.
20 19 Lesion + PPX versus Lesion + STN-DBS + PPX: $*p < .05$.

21
22
23

**Subthalamic nucleus stimulation impairs motivation: implication for apathy in
Parkinson's disease**

Yvan Vachez, PhD^{1,2}, Carole Carcenac, PhD^{1,2}, Robin Magnard, PhD^{1,2}, Lydia Kerkerian -Le Goff,
PhD³, Pascal Salin, PhD³, Marc Savasta, PhD^{1,2}, Sebastien Carnicella, PhD^{1,2} and Sabrina Boulet,
PhD^{1,2}

Affiliations :

¹Inserm, U1216, F- 38000 Grenoble, France.

²Univ. Grenoble Alpes, Grenoble Institut des Neurosciences, GIN, F-38000 Grenoble, France.

³Aix Marseille Univ, CNRS, IBDM, Marseille, France.

Corresponding authors:

Dr Sabrina Boulet and Dr Marc Savasta

INSERM U1216, Grenoble Institute of Neuroscience, Group "Pathophysiology of Motivation",
Grenoble Université - Site Santé La Tronche - BP 170, 38042 Grenoble, France. *Phone:* 33 4
56 52 06 70;

Email addresses: [Sabrina.boulet@univ-grenoble-alpes.fr](mailto: Sabrina.boulet@univ-grenoble-alpes.fr) ; [marc.savasta@inserm.fr](mailto: marc.savasta@inserm.fr)

Running title: STN-DBS and Apathy in Parkinson's Disease

Relevant conflicts of interest/financial disclosures: Nothing to report.

Number of characters in the title: 130

Number of characters in the running title: 41

Number of words in the Abstract: 250

Number of words in the body of the manuscript: 4387

Number of figures: 5

STN-DBS and Apathy in Parkinson's Disease

ABSTRACT

Background: Apathy is one of the most disabling neuropsychiatric symptoms in Parkinson's disease (PD) patients, and has a higher prevalence in patients under subthalamic nucleus deep brain stimulation (STN-DBS). Indeed, despite its effectiveness for alleviating PD motor symptoms, its neuropsychiatric repercussion has not been fully uncovered yet. Because it can be alleviated by dopaminergic therapies, especially D₂ and D₃ dopaminergic receptor (D₂R/D₃R) agonists, the commonest explanation proposed for apathy after STN-DBS is a too strong reduction of dopaminergic treatments.

Objectives: Whether or not STN-DBS can induce apathetic behaviors remains an important matter of concern. We aimed at unambiguously addressing this question of the motivational effects of chronic STN-DBS.

Methods: We longitudinally assessed the motivational effects of chronic STN-DBS, by using innovative wireless micro-stimulators allowing continuous stimulation of STN in freely moving rats, and a pharmacological therapeutic approach.

Results: We showed for the first time that STN-DBS induces a motivational deficit in naïve rats and intensifies those existing in a rodent model of PD neuropsychiatric symptoms. As reported from clinical studies, this loss of motivation was fully reversed by chronic treatment with pramipexole, a D₂R/D₃R agonist.

Conclusion: Taken together, these data provide experimental evidence that chronic STN-DBS by itself can induce a loss of motivation, reminiscent of apathy, independently of the dopaminergic neurodegenerative process or reduction of dopamine replacement therapy, presumably reflecting a dopaminergic driven deficit. Therefore, our data help to clarify and reconcile conflicting clinical observations by highlighting some of the mechanisms of the neuropsychiatric side-effects induced by chronic STN-DBS.

STN-DBS and Apathy in Parkinson's Disease

1 Introduction

2 Subthalamic nucleus deep brain stimulation (STN-DBS) is a neurosurgical treatment
3 that efficiently alleviates the motor symptoms of Parkinson's disease (PD).¹ However, a
4 plethora of psychiatric manifestations and cognitive deficits have been recently identified as
5 an integral part of the clinical picture of the disease and STN-DBS has been suggested to
6 influence these symptoms, for better or for worse.^{2, 3} Apathy, which can be simplistically
7 defined as a loss of motivation or a reduction in goal-directed behaviors accompanied by loss
8 of emotions and flattening of affect,⁴⁻⁶ is the most frequently observed non-motor
9 complication of PD and deeply contributes to worsen the patient's quality of life^{2, 5, 7-12}.
10 Importantly, apathy has been reported to occur, or be exacerbated, in some patients under
11 STN-DBS, as blunted affects^{8-10, 13-16}. Yet, the clear repercussion of STN-DBS on apathy
12 remains to be elucidated.

13 Because dopaminergic replacement therapy (DRT) is reduced during STN-DBS,
14 apathy in patients under STN-DBS is commonly attributed to the resurgence of pre-existing
15 symptoms revealed by DRT reduction or withdrawal.¹⁷ This assumption is also supported by
16 1) the alleviation of apathy after STN-DBS by dopaminergic agonists, especially those
17 targeting the D2 and D3 DA receptors (D₂R and D₃R)^{18, 19} and 2) functional imaging studies
18 in PD patients^{9, 17} and some preclinical data indicating that at least some forms of apathy are
19 related to the degenerative process and DA loss.²⁰

20 However, in several studies, the occurrence of apathy in patients under STN-DBS
21 was not correlated with the reduction of DRT but with DBS-induced changes in glucose
22 metabolism within the associative and limbic circuitry^{15, 21, 22} or with incorrect location of
23 electrodes in the associative or limbic part of the STN.²³ Thus, apathy in patients under STN-
24 DBS was also proposed to be a major side-effect of STN-DBS itself.^{15, 21-23} There is now an
25 abundance of experimental and clinical studies demonstrating that 1) the STN is involved in
26 reward and motivational processes,²⁴⁻³¹ 2) manipulating the STN can modify motivational
27 behaviors³²⁻³⁷ and 3) STN-DBS can alleviate dopamine dysregulation syndrome in some PD

STN-DBS and Apathy in Parkinson's Disease

1 patients^{14, 38, 39} and be a conceivable approach to treat addictive disorders⁴⁰⁻⁴⁵. Yet, a
2 possible direct impact of STN-DBS on motivation in PD is not unanimously supported.

3 This lack of consensus concerning the origin of apathy in patients under STN-DBS is
4 a critical issue. Whereas the prevalence of apathy before STN-DBS is about 25%^{13, 46, 47}, this
5 percentage ranges from 8 % to 60 % during STN-DBS^{14, 17, 21, 48}, according to the diagnostic
6 approach used, whether established according to cut-off scores on severity scales,
7 instruments rated by caregivers, or clinical diagnostic criteria⁴⁷. Thus, it considerably
8 compromises the benefits of STN-DBS on motor symptoms.⁵ Investigating this question in
9 patients is difficult because it is impossible to avoid the impact of the progressive process of
10 degeneration and reduction of DRT during STN-DBS. In addition, the animal studies that
11 have sought to explore the limbic and mood effects of STN-DBS may not have combined
12 appropriate behavioral approaches to assess motivation with bilateral and continuous STN-
13 DBS^{41, 49, 50} as clinically applied most of the time in studies reporting on apathy^{5, 15, 19, 21, 51-53}.

14 In the present study, we explored a potential effect of bilateral STN-DBS on
15 **motivation**, by using a wireless micro-stimulation system enabling chronic continuous
16 stimulation in rats during several weeks⁵⁴. We first investigated the consequences of bilateral
17 chronic STN-DBS in naïve rats and then in a preclinical model of neuropsychiatric symptoms
18 related to PD that we have developed, using bilateral but partial denervation of the dorsal
19 striatum (DS) by 6-hydroxydopamine (6-OHDA) lesion of SNc.^{20, 55} Because D₂R and D₃R
20 agonists, such as pramipexole (PPX), can alleviate pre and post STN-DBS apathy in PD
21 patients^{18, 19, 56} as well as in preclinical models^{20, 57} and because we reported that STN-DBS
22 reduces the level of D₂R and D₃R in the nucleus accumbens (NAc) of rats,⁵⁸ our working
23 hypothesis was that post-STN-DBS apathy may be due to an alteration of DA transmission
24 induced by STN-DBS itself. Thus, we also investigated the effect of STN-DBS with or without
25 chronic treatment with PPX.

STN-DBS and Apathy in Parkinson's Disease

1 Materials and Methods**2 Animals**

3 Experiments were performed on adult male Sprague-Dawley rats (Janvier, Le Genest-Saint-
4 Isle, France) weighing approximately 350g (8 weeks old) at the time of surgery. Animals
5 were individually housed under standard laboratory conditions (12 h light/dark cycle, with
6 lights on at 7 am) with food and water available *ad libitum* during all the experimental
7 procedures. Protocols used complied with the European Union 2010 Animal Welfare Act and
8 the French directive 2010/63.

**10 Bilateral 6-OHDA lesions, bilateral implantation of electrodes and deep brain
11 stimulation of subthalamic nucleus**

12 See supplemental informations

14 Experimental design

15 After self-administration training and both surgeries, rats were subjected to a sequence of
16 behavioral tests, with one resting day between the different tests (Figure 1A). In each
17 experiment, all conditions were counter-balanced among the different test chambers and
18 each apparatus was thoroughly cleaned after each trial or session.

19 Rats were trained to self-administer a 2.5% sucrose solution before and after SNc lesion and
20 electrodes implantation (Only the self-administration after electrode implantation is
21 represented). After 10 to 15 days, stable performances were obtained (less than 20%
22 performance variation over three consecutive sessions) and STN-DBS was turned ON.
23 Pharmacological procedures were applied after a new stabilization period: PPX (Sigma, 0.3
24 mg.kg⁻¹ in 0.9% NaCl, 1 ml/kg) or vehicle was administered (sub-cutaneous) twice a day, 3h
25 before the beginning of behavioral tests (i.e. injection at 7 am; test at 10 am) for and then at
26 5 pm, during 20 days. This protocol, known to increase the expression of D₂R and D₃R,⁵⁹
27 was chosen to explore the chronic effects of PPX.⁶⁰ STN-DBS and PPX treatment were

STN-DBS and Apathy in Parkinson's Disease

1
2
3 1 uninterrupted until rat euthanasia. After several days of sucrose self-administration, rats were
4
5 2 submitted to a two-bottle choice procedure, as well as to a stepping test and
6
7 3 locomotor/ambulatory activity evaluation in an open area⁵⁵ (Figure 1A). At the end of the
8
9 4 experiment, rats were euthanized and brains were processed for histological control of lesion
10
11 5 and implantations.
12
13 6

15 7 See supplemental data for full description of behavioral procedures, quantification of the
16
17 8 extent of the striatal DA denervation and control of electrode implantation.
18
19 9

10 ***Data and statistical analysis***

11 Data are shown as means \pm SEM and were analyzed by one or two-way ANOVAs, repeated
12
13 or not as specified in Results. Concerning operant sucrose self-administration, the different
14
15 experimental periods (Pre-STN-DBS, STN-DBS and STN-DBS + PPX) were analyzed
16
17 independently by distinct repeated measure ANOVAs for figures 2A and 4A. When indicated,
18
19 post hoc analyses were carried out with the Student Newman-Keuls procedure.
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

STN-DBS and Apathy in Parkinson's Disease

1 Results**2 *Histological controls***

3 Figure 1B provides the different electrode positions in left and right STN for the
4 stimulated animals. Figures 1C and D illustrate two examples of the position of an electrode
5 tip within the STN, stimulated respectively with the lowest (100 μ A) and the highest (225 μ A)
6 intensity used in the study. See also supplemental figure 1 for additional examples of STN
7 stimulated at 100 μ A or 225 μ A, still unaltered after chronic stimulation.

8 Bilateral lesion of SNc (Figure 1E, percent of TH-IR loss, left SNc: 73 ± 5 ; right SNc:
9 74 ± 5) was obtained by 6-OHDA injection. The injection produced an important denervation
10 of the dorsal striatum in its lateral portion (Figure 1F, left dorsal striatum: 68 ± 5 ; right dorsal
11 striatum: 74 ± 6), along its rostro-caudal extent as revealed by decreased tyrosine
12 hydroxylase immunoreactivity. As the lesion has been shown to specifically affect SNc,
13 barely impacting VTA (Figure 1G, left VTA: 11 ± 8 ; right VTA: 20 ± 4 ; Two way ANOVA,
14 Structure x Lesion interaction: $F_{1,22} = 7.654$, $p < .0151$), NAc was almost totally preserved
15 from denervation (Figure 1H, left NAc: 21 ± 3 ; right NAc: 24 ± 4 ; Two way ANOVA, Structure
16 x Lesion interaction: $F_{1,22} = 125.758$, $p < .001$).

18 *STN-DBS induces a motivational deficit in normal rats that is reversed by the D₂R/D₃R*
19 *agonist pramipexole*

20 Before STN-DBS was switched ON, rats were trained for 10 days in the operant task
21 (only the last 3 days of training are represented in figure 2A). Groups were formed to have
22 equivalent performance levels (Figure 2A, Pre STN-DBS period, repeated measure ANOVA,
23 Session x STN-DBS x PPX interaction, $F_{2,122} = 1.334$, $p = .2672$). From day one of
24 stimulation, STN-DBS induced a dramatic decrease of about 40% in instrumental responding
25 for the sucrose solution in both stimulated groups before pharmacological treatment (STN-
26 DBS + Veh and STN-DBS + PPX) as compared with the pre STN-DBS levels (Figure 2A,
27 STN-DBS period, repeated measure ANOVA, STN-DBS effect: $F_{1,54} = 9.615$, $p = .0031$;

STN-DBS and Apathy in Parkinson's Disease

1
2
3 1 Figure S2A, two way ANOVA, STN-DBS effect: $F_{1,42} = 10.480$, $p = .002$). PPX completely
4
5 2 rescued the self-administration performances of stimulated rats from the second day of
6
7 3 treatment, without bringing performances superior to baseline or to Control + Veh rat levels.
8
9 4 (Figure 2A, STN-DBS + PPX period, repeated measure ANOVA, STN-DBS x PPX
10
11 5 interaction: $F_{1,58} = 4.658$, $p = .0351$; Session x PPX interaction: $F_{6,348} = 2.412$, $p = .0269$).
12
13 6 Both STN-DBS and PPX effects remained stable until the end of self-administration
14
15 7 experimentation (Figure 2B, two ways ANOVA, STN-DBS x PPX interaction: $F_{1,64} = 7.178$, p
16
17 8 = .009). In order to determine whether the decrease of operant performances induced by
18
19 9 STN-DBS was due to impairment in the consummatory or preparatory components of
20
21 10 motivated behaviors, we evaluated the sensitivity to the motivational properties of sucrose
22
23 11 (consummatory behavior) in a two-bottle choice procedure. The preference for the sucrose
24
25 12 solution that was used in the operant self-administration experiment over water was high and
26
27 13 not altered by STN-DBS or PPX (Figure 2C, two way ANOVA, STN-DBS x PPX interaction:
28
29 14 $F_{1,61} = .966$, $p = .330$). Therefore, the self-administration paradigm and the sucrose
30
31 15 preference test indicate that STN-DBS affected the preparatory component of motivated
32
33 16 behaviors during the operant task and that PPX specifically corrected this impairment.

34
35
36 17 In addition, STN-DBS-induced preparatory component deficit and subsequent self-
37
38 18 administration deficiency cannot be attributed to motor impairment, because STN-DBS did
39
40 19 not induce any significant locomotor change during the first 20 minutes of the open area test,
41
42 20 corresponding to the exploratory phase when locomotor activity is high, facilitating detection
43
44 21 of motor deficit (Figure 3A, two way ANOVA, 0-20 min: $F_{1,48} = .513$; $p = .477$). Exploration
45
46 22 and locomotor activity then progressively decreased to a basal level during the last 2 periods
47
48 23 (20 to 60 min). Although it did not reach significance, STN-DBS tended to reduce ambulatory
49
50 24 activity during this phase. (Figure 3A, STN-DBS effect, Two way ANOVA, 20-40 min: $F_{1,48} =$
51
52 25 3.311 , $p = .075$; 40-60 min: $F_{1,48} = 3.525$, $p = .067$) This effect of STN-DBS is unlikely to
53
54 26 reflect a motor deficit but rather a decrease in general behavioral activity, because
55
56 27 ambulatory speed during this test phase was not changed by the stimulation (Figure 3B,
57
58 28 STN-DBS effect, Two way ANOVA, $F_{1,48} = .196$, $p = .660$). Moreover, fine motor skills of left

STN-DBS and Apathy in Parkinson's Disease

1 and right limbs assessed using a stepping test were not affected by the stimulation,
2 confirming the absence of any motor deficit induced by chronic and bilateral STN-DBS
3 (Figure 3C). As STN-DBS, PPX had no impact on fine motor skills (Figure 3C, two way
4 ANOVA, STN-DBS x PPX interaction: left to right moves, left limb: $F_{1,55} = .110$, $p = .742$; right
5 limb $F_{1,55} = .000442$, $p = .983$; right to left moves, left limb: $F_{1,55} = .0135$, $p = .908$; right limb:
6 $F_{1,55} = .0557$, $p = .814$), but caused a significant increase in locomotor activity in the open
7 area test (Figure 3A, PPX effect, Two way ANOVA, 20-40 min: $F_{1,48} = 5.686$, $p = .021$; 40-60
8 min: $F_{1,48} = 5.047$, $p = .030$). Interestingly, the combined effect of the behavioral hypoactivity
9 induced by STN-DBS and hyperactivity due to PPX resulted in normalization of the general
10 activity during the last 2 phases of the open area test (Figure 3A).

11 Taken together, these results suggest that STN-DBS induced a clear motivational
12 deficit that was alleviated by the D_2R/D_3R agonist, reminiscent of apathy in PD.

13
14 ***STN-DBS exacerbates motivational deficit in a rodent model of PD apathetic
15 symptoms and affective disorders***

16 We sought to confirm whether STN-DBS would also affect motivation in a
17 pathological context. We used a rodent model of PD that we developed⁵⁵ and that exhibits
18 strong motivational deficits, without locomotor alterations.

19 Before STN-DBS was switched-ON, rats were trained again for 10 days in the
20 operant task (only the last 3 days of training are represented in figure 4A). All groups
21 obtained equivalent performance levels (Figure 4A, 3 last days of Pre STN-DBS period,
22 repeated measure ANOVA, Session x STN-DBS x PPX interaction, $F_{2,32} = 1.088$, $p = .3490$).
23 As previously demonstrated,⁵⁵ 6-OHDA rats showed a marked and significant decrease in
24 self-administration of sucrose as compared to non-lesioned animals (Figure S2A, two way
25 ANOVA, Lesion effect: $F_{1,42} = 26.592$, $p = < .001$; Figure S2B, two way ANOVA, Lesion
26 effect: $F_{1,50} = 26.368$, $p = < .001$) which was not reversed by PPX treatment alone (Figure
27 S2B, two way ANOVA, PPX effect: $F_{1,50} = .150$, $p = .701$). Despite this important instrumental
28 deficit induced by the lesion, STN-DBS was still able to reduce operant behavior by about

STN-DBS and Apathy in Parkinson's Disease

1
2
3 1 50% from day one of stimulation (Figure 4A, STN-DBS period, repeated measure ANOVA,
4
5 2 STN-DBS effect $F_{1,15} = 4.249$, $p = .0571$), as observed in non-lesioned/control stimulated rats
6
7 3 (Figure S2A, two way ANOVA, STN-DBS effect: $F_{1,42} = 10.48$, $p = .002$ and no STN-DBS x
8
9 4 Lesion interaction: $F_{1,42} = 1,112$, $p = .298$). As for non-lesioned animals, PPX completely
10
11 5 alleviated this STN-DBS-induced deficit from the second day of treatment (STN-DBS + PPX
12
13 6 period, repeated measure ANOVA, STN-DBS x PPX x Session interaction effect $F_{9,108} =$
14
15 7 2.712 , $p = .0069$). Both STN-DBS and PPX effects remained stable until the end of the
16
17 8 experiment (Figure 4B, two way ANOVA, STN-DBS x PPX interaction: $F_{1,16} = .826$, $p = .377$).
18
19 9 We also assessed the consummatory components of motivated behaviors, with the two-
20
21 10 bottle choice procedure and we found that neither STN-DBS nor PPX modified the
22
23 11 preference for sucrose (Figure 4C, two way ANOVA, STN-DBS x PPX interaction: $F_{1,20} =$
24
25 12 $.341$, $p = .567$).

26
27
28 13 PPX or STN-DBS tended to reduce ambulatory activity in the open area test while
29
30 14 when they were combined, on average they promoted a high level of activity but with high
31
32 15 variability (Figure 5A, two way ANOVA, STN-DBS x PPX interaction: $F_{1,14} = 5.759$, $p = .031$).
33
34 16 These effects are likely to reflect changes in behavioral activity rather than alteration of
35
36 17 motor skills *per se*, first because neither PPX nor STN-DBS affected the ambulatory speed
37
38 18 during the open area test (Figure 5B, Two way ANOVA, STN-DBS x PPX interaction $F_{1,48} =$
39
40 19 $.214$, $p = .650$) and second, adjustments during the stepping test were not impacted by either
41
42 20 treatment (Figure 5B, two ways ANOVA, STN-DBS x PPX interaction: left to right moves, left
43
44 21 limb: $F_{1,18} = .184$, $p = .673$; right limb: $F_{1,18} = .653$, $p = .983$; right to left moves, left limb: $F_{1,18}$
45
46 22 $= .0017$, $p = .915$; right limb: $F_{1,18} = .764$, $p = .393$).

47
48
49 23 Taken together, these results suggest that STN-DBS exacerbates the loss of
50
51 24 motivation induced by the dopaminergic lesion and reduce behavioral activity, effects that
52
53 25 were alleviated by PPX.
54
55
56 26
57
58 27
59
60

STN-DBS and Apathy in Parkinson's Disease

Discussion

Combining a relevant *in vivo* model of neuropsychiatric PD symptoms, chronic bilateral STN-DBS in awake and freely moving animals and a pharmacological approach, we provide strong evidence for a direct deleterious effect of STN-DBS on motivation. STN-DBS induces a significant deficit in motivated behavior, due neither to a reward sensitivity deficit related to a potential anhedonic effect, nor to motor deficits. This behavioral effect was completely reversed by the activation of D₂R/D₃R with PPX, revealing the potential critical role of DA in this STN-DBS motivational effect.

It is essential to better understand the pathophysiology of neuropsychiatric symptoms of PD and how they respond to current treatments such as STN-DBS. Here, we tried to be as close as possible to the clinical situation in PD, especially for the stimulation conditions. While most preclinical studies have been performed with acute STN-DBS that was applied daily only during the tasks and experiments (e.g.,^{41, 49}), the novel stimulation system we used allowed continuous STN-DBS for several days. In addition, we used monopolar stimulation. Indeed, it is the most widely applied in PD patients¹ as well as in studies describing apathy under STN-DBS^{17, 21, 48, 61-64}. Furthermore, a preclinical study in rat has clearly demonstrated that it reduces tissue damage compared to bipolar stimulation.⁶⁵ Moreover, although monopolar stimulation is more likely to affect the surrounding STN fibers than bipolar stimulation, it was applied at intensities below 225 μ A to minimize current spreading as previously demonstrated.⁶⁶ The motivational dimension of apathy especially concerns hum-drum daily tasks that are neither challenging nor particularly effortful.⁴ A sucrose self-administration procedure with a 2.5 % solution, in a fixed ratio 1 schedule, in non-food-deprived rats, is an appropriate way to operationalize a simple effort with a relatively moderate rewarding outcome. This approach has allowed us to demonstrate a clear effect of STN-DBS on motivated behaviors during an operant task. The decrease of baseline ambulatory activity detected during the open area test, unrelated to motor impairment, further suggests that the STN-DBS-induced operant deficit may be considered as a decrease in the maintenance of

STN-DBS and Apathy in Parkinson's Disease

1 behavioral activity, as observed in apathy.⁹ While we previously provided strong evidence
2 that the dopaminergic denervation can account for the loss of motivation in PD, here we
3 report that STN-DBS by itself could induce or exacerbate **this motivational impairment** via
4 DA-driven mechanisms, independent of the hypodopaminergy induced by DRT reduction.
5 Furthermore, the present study provides a missing link, reconciling apparently contradictory
6 clinical observations on the origin of post-operative apathy in stimulated PD patients.

7 The myriad of reported neuropsychiatric side effects of STN-DBS in PD patients³ has
8 triggered several animal studies to understand their phenomenology. These studies have
9 demonstrated the possible implication of STN-DBS in PD-related depression^{49, 67} or potent
10 effects on impulsive behaviors,⁶⁸ but few of them tackled the effects on pure motivational
11 processes. As far as we know, only one report mentions data that could be interpreted as a
12 transient reduced motivation under STN-DBS.⁶⁹ However, another study showed no effect of
13 STN-DBS on operant activity in a fixed-ratio schedule of reinforcement and even described
14 an increase in performance in obtaining a sucrose pellet during a progressive ratio
15 schedule.⁴¹ Major differences between this previous study and our protocol are likely to
16 account for these discrepancies. Indeed, Rouaud et al., used acute bipolar stimulation
17 conditions compared to our chronic monopolar STN-DBS; interestingly, psychiatric side
18 effects of STN-DBS strongly depend on electrode polarity. For example, acute depressive
19 states following regular monopolar STN-DBS are greatly reduced by switching to the scarcer
20 bipolar STN-DBS.^{70, 71} Moreover, the design of the operant task was different enough to yield
21 diverging results. In particular, our study avoided food-restriction. Whereas all the studies
22 having inactivated the STN found an increase in motivation for food in restricted animals, one
23 study demonstrated that this effect was completely occluded in rats fed *ad libitum*.³⁷ Finally,
24 while the conditioned place preference revealed an effect of STN-DBS on the rewarding
25 properties of sucrose in this study,⁴¹ we did not find any modification of this parameter during
26 the two bottle choice test.

27 The STN can be functionally and anatomically subdivided into motor, associative and
28 limbic sub-territories.⁷²⁻⁷⁴ Then it is part of the three respective basal ganglia loops.^{73, 74} In PD

STN-DBS and Apathy in Parkinson's Disease

1 patients, DBS is tailored to stimulate the motor part of the STN, and so to impact the whole
2 motor circuitry. However, an inaccurate electrode placement within the STN can engage the
3 circuitries involving its associative or limbic part rather than the motor one,⁷⁵ potentially
4 leading to non-motor side effects,^{70, 74} apathy in particular. In line with this assumption, a very
5 recent study succeeded in alleviating post STN-DBS apathy in patients by displacing the
6 electrode from the limbic STN to the motor part,²³ demonstrating that apathy under STN-DBS
7 could rely on a mechanism caused by the stimulation of the limbic part of the STN. Our data
8 suggest that the dopaminergic system might be involved in this mechanism, at least in part.

9 In patients, apathy occurring after STN-DBS can be treated by dopaminergic
10 agonists, including those targeting the D₂R and D₃R.^{18, 19} This, and the fact that
11 methylphenidate can alleviate fatigue⁷⁶, which is a related and/or confounding symptom of
12 apathy⁷⁷, has prompted the hypothesis that a strong hypodopaminergic state revealed by
13 reduction of DRT, rather than STN-DBS *per se*, may be responsible for the resurgence of
14 apathy.¹³ Using a pharmacological protocol allowing exploration of the chronic effects of PPX
15 and known to increase D₂R and D₃R expression,⁵⁹ we demonstrated that PPX, a D₂R/D₃R
16 agonist, fully rescued the motivational deficit induced by STN-DBS in control and lesioned
17 rats. It should be noted that this pharmacological protocol was not able to reverse the loss of
18 motivation due to the dopaminergic lesion, indicating that even if both the lesion and STN-
19 DBS effects on motivation could be driven by dopaminergic mechanisms, these could be
20 significantly different. This suggests that STN-DBS may impact on behavior via its own
21 effects on DA transmission, which is consistent with previous animal model studies showing
22 that STN-DBS modulates DA system.^{58, 78-82} The three so-called basal ganglia limbic,
23 associative and motor loops including the STN are not completely segregated; the different
24 structures involved are functionally interconnected⁸³ by an ascending striato-midbrain-striatal
25 spiraling circuitry.⁸⁴ Moreover, beyond this complex organization, which has yet to be
26 demonstrated in rats, SNc and VTA are overlapping structures⁸⁵ and the whole striatum is
27 characterized by a dense local microcircuitry^{86, 87} which could also contribute to this
28 interconnection. Thus, STN-DBS may modulate DA transmission within the basal ganglia

STN-DBS and Apathy in Parkinson's Disease

1 and, through these subcortical interactions, disrupt the complex chain of events that
2 transforms intentions into adapted action, and thereby influence non-motor functions.^{6, 9}
3 Nonetheless, we cannot exclude a potential involvement of the structures surrounding the
4 STN . It is unlikely that the electric field emanating from the electrode is restrained to the
5 STN borders and DBS of some of these structures in the vicinity of the STN, such as the
6 lateral hypothalamus, can also modulate DA transmission⁸⁸. We previously found that short
7 STN-DBS can decrease the level of D₂R and D₃R within the NAc.⁵⁸ Given the critical
8 implication of the D₂R and D₃R in motivational processes,⁸⁹⁻⁹² our data suggest a causal
9 involvement of these receptors in the motivational deficit that we observed. In addition, D₂R
10 and D₃R are expressed in several other non-motor structures impacted by PPX treatment⁹³
11 and some of them are part of down-stream circuits with metabolic activities known to be
12 affected by STN-DBS.^{22, 94} Thus, beyond the NAc, both STN-DBS and PPX effects could
13 differentially engage some common system responsible for behavior changes highlighted in
14 this study. However, dopaminergic agonists do not always efficiently alleviate apathy under
15 STN-DBS²². Non dopaminergic lesions frequently occur during the course of disease^{95, 96},
16 and serotonin for example is proposed to participate in PD apathy⁹⁷. In rats, STN-DBS has
17 been shown to decrease serotonin release promoting depressive like behavior.^{49, 98} Thus,
18 we cannot completely exclude the implication of other neurotransmitters in the loss of
19 motivation observed in patients or in this study.

20 As previously reported,⁹⁹ PPX at 0.3 mg/kg induces hyperlocomotricity in the open
21 area test in control rats. This hyperactivity is unlikely to be responsible for the reversion of
22 the self-administration deficit observed in PPX-treated STN-DBS rats since first, the level of
23 non-stimulated treated rats remains similar to that of control rats, and second, PPX induced
24 hypoactivity in non-stimulated lesioned rats whereas it also reversed the operant deficit
25 induced by STN-DBS in those lesioned rats. This hypoactivity promoted by PPX in lesioned
26 rats has previously been reported during the 120 minutes post injection window in control
27 animals⁹⁹ and could rely on a sedating effect mediated by the presynaptic D₃R¹⁰⁰. We chose
28 to separate the behavioral test from injection by 3h to avoid this effect. Since we previously

STN-DBS and Apathy in Parkinson's Disease

1 demonstrated that the 6-OHDA lesion modifies D₃R expression within the dorsolateral part of
2 the striatum¹⁰¹, local imbalance between pre and post synaptic D₂R and/or D₃R expression
3 or activity¹⁰² could be responsible for DA transmission blunting, thus promoting this
4 hypoactivity. Very intriguingly, in some rats of both the control and lesioned groups, the
5 combination of STN-DBS and PPX induced a very high- level of activity that could emphasize
6 the importance of individual traits, as demonstrated for the expression or severity of impulse
7 control disorder in PD¹⁰³⁻¹⁰⁵ or compulsive drug taking.¹⁰⁶ Thus, depending on complex
8 interactions with dopaminergic treatments and according to pre-existing individual traits,
9 STN-DBS could also lead to the development of hyperdopaminergic states reminiscent of
10 hypomanic behaviors also observed in PD patients under STN-DBS.^{62, 74, 107, 108}

11 Altogether, these data bring coherence to clinical observations that seemed
12 contradictory until today: apathy in PD patients, at least its motivational dimension, could be
13 induced by STN-DBS itself and attenuated by activation of D₂R/D₃R, regardless of (or in
14 addition to) DRT reduction and the DA-mediated hypofunction. More detailed molecular
15 analysis or techniques such as optogenetics could enable us to clarify the structures or sub-
16 territories involved in this STN-DBS induced DA-driven loss of motivation. While it is beyond
17 the scope of the present study, it would be also of interest to further investigate the effect of
18 STN-DBS on the emotional and affective component of apathy, as STN has been proposed
19 to be involved in such processes¹⁰⁹.

20 STN-DBS has been shown to prevent cocaine or heroin re-escalation intake in rats⁴⁰,
21⁴⁴ and to reduce dopamine dysregulation syndrome or addictive behavior such as
22 pathological gambling.¹¹⁰⁻¹¹⁴ Thus, STN-DBS is currently explored as an effective treatment
23 for addiction.^{45, 115} However, our results suggest that this apparent beneficial effect could be
24 underlain by a general motivational deficit. In rats, inhibiting the STN can abolish affective
25 responses for salient reward¹⁰⁹ and STN-DBS can induce depression-like behaviors.^{49, 67, 116}
26 Furthermore, PD patients for whom addictive behavior was reduced under STN-DBS can
27 also co-express apathy.^{14, 110} These convergent facts are raising the question of the nature of
28 this apparent "anti-addictive" effect of STN-DBS. This neurosurgical treatment could rather

STN-DBS and Apathy in Parkinson's Disease

1
2
3 1 induce a state of negative affect, comparable to a certain extent to the blunted dopaminergic
4
5 2 transmission observed during prolonged drug consumption¹¹⁷⁻¹¹⁹ , increasing the risk of
6
7 3 addictive behavior resurgence in the same way as drugs of abuse withdrawal syndrome
8
9 4 promotes relapse.¹²⁰
10

11 5 Thus, this new insight calls for reconsideration of the role of STN-DBS on mood in PD
12
13 6 to improve patient care and quality of life, and more broadly, to rethink the use of STN-DBS
14
15 7 in psychiatry.
16
17
18 8
19
20 9
21
22 10
23
24 11
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

STN-DBS and Apathy in Parkinson's Disease

1 Financial disclosure

2 This work was supported by the Institut National de la Santé et de la recherche Médicale,
3 Fondation de France, Région Rhône-Alpes (Arc n°2), Neurodis, Grenoble Alpes Université,
4 CNRS, Aix-Marseille Université and France Parkinson. The authors declare no conflict of
5 interest.

7 Acknowledgments

8 We would like to thank Fiona Hemming for her critical reading, as well as Séverine Lirzin and
9 Olivier Mainard for the maintenance and their technical help concerning the micro-stimulator
10 system.

12 Author contributions

13 YV, SB, SC and MS were responsible for the conception and the design of the study. YV,
14 SB, CC and RM carried out the stereotaxic surgeries and the post-surgery monitoring of the
15 animals. YV, CC and SB performed the neuroanatomical analysis of electrode implantation
16 and lesions. YV and SB carried out the behavioral experiments and analysis. LKLG and PS
17 provided micro-stimulators as well as training and advice for their use. YV and SB wrote the
18 paper with the help of the other authors.

19
20
21 Supplementary information accompanies this paper.

STN-DBS and Apathy in Parkinson's Disease

References

1. Benabid AL, Chabardes S, Mitrofanis J, Pollak P. Deep brain stimulation of the subthalamic nucleus for the treatment of Parkinson's disease. *Lancet Neurol* 2009;8(1):67-81.
2. Chaudhuri KR, Schapira AH. Non-motor symptoms of Parkinson's disease: dopaminergic pathophysiology and treatment. *Lancet Neurol* 2009;8(5):464-474.
3. Castrioto A, Lhommee E, Moro E, Krack P. Mood and behavioural effects of subthalamic stimulation in Parkinson's disease. *Lancet Neurol* 2014;13(3):287-305.
4. Marin RS. Apathy: Concept, Syndrome, Neural Mechanisms, and Treatment. *Semin Clin Neuropsychiatry* 1996;1(4):304-314.
5. Martinez-Fernandez R, Pelissier P, Quesada JL, et al. Postoperative apathy can neutralise benefits in quality of life after subthalamic stimulation for Parkinson's disease. *Journal of neurology, neurosurgery, and psychiatry* 2016;87(3):311-318.
6. Levy R, Dubois B. Apathy and the functional anatomy of the prefrontal cortex-basal ganglia circuits. *Cereb Cortex* 2006;16(7):916-928.
7. Chagraoui A, Boukharz L, Thibaut F, Anouar Y, Maltete D. The pathophysiological mechanisms of motivational deficits in Parkinson's disease. *Progress in neuro-psychopharmacology & biological psychiatry* 2018;81:138-152.
8. Chaudhuri KR, Odin P. The challenge of non-motor symptoms in Parkinson's disease. *Prog Brain Res* 2010;184:325-341.
9. Pagonabarraga J, Kulisevsky J, Strafella AP, Krack P. Apathy in Parkinson's disease: clinical features, neural substrates, diagnosis, and treatment. *Lancet Neurol* 2015;14(5):518-531.
10. Sierra M, Carnicella S, Strafella AP, et al. Apathy and Impulse Control Disorders: Yin & Yang of Dopamine Dependent Behaviors. *Journal of Parkinson's disease* 2015;5(3):625-636.
11. Maier F, Lewis CJ, Horstkoetter N, et al. Subjective perceived outcome of subthalamic deep brain stimulation in Parkinson's disease one year after surgery. *Parkinsonism & related disorders* 2016;24:41-47.
12. Maier F, Lewis CJ, Horstkoetter N, et al. Patients' expectations of deep brain stimulation, and subjective perceived outcome related to clinical measures in Parkinson's disease: a mixed-method approach. *Journal of neurology, neurosurgery, and psychiatry* 2013;84(11):1273-1281.
13. Aarsland D, Bronnick K, Alves G, et al. The spectrum of neuropsychiatric symptoms in patients with early untreated Parkinson's disease. *Journal of neurology, neurosurgery, and psychiatry* 2009;80(8):928-930.
14. Abbes M, Lhommee E, Thobois S, et al. Subthalamic stimulation and neuropsychiatric symptoms in Parkinson's disease: results from a long-term follow-up cohort study. *Journal of neurology, neurosurgery, and psychiatry* 2018;89(8):836-843.
15. Drapier D, Peron J, Leray E, et al. Emotion recognition impairment and apathy after subthalamic nucleus stimulation in Parkinson's disease have separate neural substrates. *Neuropsychologia* 2008;46(11):2796-2801.
16. Witt K, Daniels C, Reiff J, et al. Neuropsychological and psychiatric changes after deep brain stimulation for Parkinson's disease: a randomised, multicentre study. *Lancet Neurol* 2008;7(7):605-614.
17. Thobois S, Ardouin C, Lhommee E, et al. Non-motor dopamine withdrawal syndrome after surgery for Parkinson's disease: predictors and underlying mesolimbic denervation. *Brain : a journal of neurology* 2010;133(Pt 4):1111-1127.

STN-DBS and Apathy in Parkinson's Disease

18. Thobois S, Lhomme E, Klinger H, et al. Parkinsonian apathy responds to dopaminergic stimulation of D2/D3 receptors with piribedil. *Brain : a journal of neurology* 2013;136(Pt 5):1568-1577.
19. Czernecki V, Schupbach M, Yaici S, et al. Apathy following subthalamic stimulation in Parkinson disease: a dopamine responsive symptom. *Movement disorders : official journal of the Movement Disorder Society* 2008;23(7):964-969.
20. Magnard R, Vachez Y, Carcenac C, et al. What can rodent models tell us about apathy and associated neuropsychiatric symptoms in Parkinson's disease? *Transl Psychiatry* 2016;6:e753.
21. Drapier D, Drapier S, Sauleau P, et al. Does subthalamic nucleus stimulation induce apathy in Parkinson's disease? *Journal of neurology* 2006;253(8):1083-1091.
22. Le Jeune F, Drapier D, Bourguignon A, et al. Subthalamic nucleus stimulation in Parkinson disease induces apathy: a PET study. *Neurology* 2009;73(21):1746-1751.
23. Zoon TJ, de Bie RM, Schuurman PR, van den Munckhof P, Denys D, Figeet M. Resolution of apathy after dorsal instead of ventral subthalamic deep brain stimulation for Parkinson's disease. *Journal of neurology* 2019.
24. Breyse E, Pelloux Y, Baunez C. The Good and Bad Differentially Encoded within the Subthalamic Nucleus in Rats(1,2,3). *eNeuro* 2015;2(5).
25. Darbaky Y, Baunez C, Arecchi P, Legallet E, Apicella P. Reward-related neuronal activity in the subthalamic nucleus of the monkey. *Neuroreport* 2005;16(11):1241-1244.
26. Espinosa-Parrilla JF, Baunez C, Apicella P. Modulation of neuronal activity by reward identity in the monkey subthalamic nucleus. *The European journal of neuroscience* 2015;42(1):1705-1717.
27. Lardeux S, Pernaud R, Paleressompoulle D, Baunez C. Beyond the reward pathway: coding reward magnitude and error in the rat subthalamic nucleus. *J Neurophysiol* 2009;102(4):2526-2537.
28. Montanari C, Giorla E, Pelloux Y, Baunez C. Subthalamic nucleus mediates the modulation on cocaine self-administration induced by ultrasonic vocalization playback in rats. *Addiction biology* 2018.
29. Lardeux S, Paleressompoulle D, Pernaud R, Cador M, Baunez C. Different populations of subthalamic neurons encode cocaine vs. sucrose reward and predict future error. *J Neurophysiol* 2013;110(7):1497-1510.
30. Justin Rossi P, Peden C, Castellanos O, Foote KD, Gunduz A, Okun MS. The human subthalamic nucleus and globus pallidus internus differentially encode reward during action control. *Human brain mapping* 2017;38(4):1952-1964.
31. Zenon A, Duclos Y, Carron R, et al. The human subthalamic nucleus encodes the subjective value of reward and the cost of effort during decision-making. *Brain : a journal of neurology* 2016;139(Pt 6):1830-1843.
32. Baunez C, Amalric M, Robbins TW. Enhanced food-related motivation after bilateral lesions of the subthalamic nucleus. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2002;22(2):562-568.
33. Baunez C, Dias C, Cador M, Amalric M. The subthalamic nucleus exerts opposite control on cocaine and 'natural' rewards. *Nat Neurosci* 2005;8(4):484-489.
34. Bezzina G, Boon FS, Hampson CL, et al. Effect of quinolinic acid-induced lesions of the subthalamic nucleus on performance on a progressive-ratio schedule of reinforcement: a quantitative analysis. *Behavioural brain research* 2008;195(2):223-230.
35. Uslaner JM, Dell'Orco JM, Pevzner A, Robinson TE. The influence of subthalamic nucleus lesions on sign-tracking to stimuli paired with food and drug rewards: facilitation of incentive salience attribution? *Neuropsychopharmacology* 2008;33(10):2352-2361.

STN-DBS and Apathy in Parkinson's Disease

- 1 36. Maillard CA, Edwards DA. [Subthalamic lesions eliminate sexual behavior in the
2 male rat]. *Journal de physiologie* 1988;83(1):50-55.
- 3 37. Pratt WE, Choi E, Guy EG. An examination of the effects of subthalamic nucleus
4 inhibition or mu-opioid receptor stimulation on food-directed motivation in the non-deprived
5 rat. *Behavioural brain research* 2012;230(2):365-373.
- 6 38. Eusebio A, Witjas T, Cohen J, et al. Subthalamic nucleus stimulation and compulsive
7 use of dopaminergic medication in Parkinson's disease. *Journal of neurology, neurosurgery,
8 and psychiatry* 2013;84(8):868-874.
- 9 39. Lhomme E, Klinger H, Thobois S, et al. Subthalamic stimulation in Parkinson's
10 disease: restoring the balance of motivated behaviours. *Brain : a journal of neurology*
11 2012;135(Pt 5):1463-1477.
- 12 40. Pelloux Y, Degoulet M, Tiran-Cappello A, et al. Subthalamic nucleus high frequency
13 stimulation prevents and reverses escalated cocaine use. *Molecular psychiatry*
14 2018;23(12):2266-2276.
- 15 41. Rouaud T, Lardeux S, Panayotis N, Paleressompouille D, Cador M, Baunez C.
16 Reducing the desire for cocaine with subthalamic nucleus deep brain stimulation. *Proceedings*
17 *of the National Academy of Sciences of the United States of America* 2010;107(3):1196-
18 1200.
- 19 42. Adams WK, Vonder Haar C, Tremblay M, et al. Deep-Brain Stimulation of the
20 Subthalamic Nucleus Selectively Decreases Risky Choice in Risk-Preferring Rats. *eNeuro*
21 2017;4(4).
- 22 43. Hachem-Delaunay S, Fournier ML, Cohen C, et al. Subthalamic nucleus high-
23 frequency stimulation modulates neuronal reactivity to cocaine within the reward circuit.
24 *Neurobiol Dis* 2015;80:54-62.
- 25 44. Wade CL, Kallupi M, Hernandez DO, et al. High-Frequency Stimulation of the
26 Subthalamic Nucleus Blocks Compulsive-Like Re-Escalation of Heroin Taking in Rats.
27 *Neuropsychopharmacology* 2017;42(9):1850-1859.
- 28 45. Creed M. Current and emerging neuromodulation therapies for addiction: insight from
29 pre-clinical studies. *Curr Opin Neurobiol* 2018;49:168-174.
- 30 46. Barone P, Antonini A, Colosimo C, et al. The PRIAMO study: A multicenter
31 assessment of nonmotor symptoms and their impact on quality of life in Parkinson's disease.
32 *Movement disorders : official journal of the Movement Disorder Society* 2009;24(11):1641-
33 1649.
- 34 47. Pagonabarraga J, Kulisevsky J. Apathy in Parkinson's Disease. *International review of*
35 *neurobiology* 2017;133:657-678.
- 36 48. Castelli L, Perozzo P, Zibetti M, et al. Chronic deep brain stimulation of the
37 subthalamic nucleus for Parkinson's disease: effects on cognition, mood, anxiety and
38 personality traits. *European neurology* 2006;55(3):136-144.
- 39 49. Temel Y, Boothman LJ, Blokland A, et al. Inhibition of 5-HT neuron activity and
40 induction of depressive-like behavior by high-frequency stimulation of the subthalamic
41 nucleus. *Proc Natl Acad Sci U S A* 2007;104(43):17087-17092.
- 42 50. Temel Y, Tan S, Vlamings R, et al. Cognitive and limbic effects of deep brain
43 stimulation in preclinical studies. *Front Biosci* 2009;14:1891-1901.
- 44 51. Czernecki V, Pillon B, Houeto JL, et al. Does bilateral stimulation of the subthalamic
45 nucleus aggravate apathy in Parkinson's disease? *Journal of neurology, neurosurgery, and*
46 *psychiatry* 2005;76(6):775-779.
- 47 52. Robert GH, Le Jeune F, Lozachmeur C, et al. Preoperative factors of apathy in
48 subthalamic stimulated Parkinson disease: a PET study. *Neurology* 2014;83(18):1620-1626.

STN-DBS and Apathy in Parkinson's Disease

- 1
2
3 1 53. Wang Y, Li Y, Zhang X, Xie A. Apathy following Bilateral Deep Brain Stimulation of
4 2 Subthalamic Nucleus in Parkinson's Disease: A Meta-Analysis. *Parkinsons Dis*
5 3 2018;2018:9756468.
6 4 54. Forni C, Mainard O, Melon C, Goguenheim D, Kerkerian-Le Goff L, Salin P. Portable
7 5 microstimulator for chronic deep brain stimulation in freely moving rats. *Journal of*
8 6 *neuroscience methods* 2012;209(1):50-57.
9 7 55. Drui G, Carnicella S, Carcenac C, et al. Loss of dopaminergic nigrostriatal neurons
10 8 accounts for the motivational and affective deficits in Parkinson's disease. *Mol Psychiatry*
11 9 2014;19(3):358-367.
12 10 56. Leentjens AF, Koester J, Fruh B, Shephard DT, Barone P, Houben JJ. The effect of
13 11 pramipexole on mood and motivational symptoms in Parkinson's disease: a meta-analysis of
14 12 placebo-controlled studies. *Clin Ther* 2009;31(1):89-98.
15 13 57. Favier M, Duran T, Carcenac C, Drui G, Savasta M, Carnicella S. Pramipexole
16 14 reverses Parkinson's disease-related motivational deficits in rats. *Movement disorders :*
17 15 *official journal of the Movement Disorder Society* 2014;29(7):912-920.
18 16 58. Carcenac C, Favier M, Vachez Y, et al. Subthalamic deep brain stimulation differently
19 17 alters striatal dopaminergic receptor levels in rats. *Movement disorders : official journal of the*
20 18 *Movement Disorder Society* 2015;30(13):1739-1749.
21 19 59. Tokunaga N, Choudhury ME, Nishikawa N, et al. Pramipexole upregulates dopamine
22 20 receptor D(2) and D(3) expression in rat striatum. *J Pharmacol Sci* 2012;120(2):133-137.
23 21 60. Micallef J, Rey M, Eusebio A, et al. Antiparkinsonian drug-induced sleepiness: a
24 22 double-blind placebo-controlled study of L-dopa, bromocriptine and pramipexole in healthy
25 23 subjects. *Br J Clin Pharmacol* 2009;67(3):333-340.
26 24 61. Castelli L, Lanotte M, Zibetti M, et al. Apathy and verbal fluency in STN-stimulated
27 25 PD patients. An observational follow-up study. *Journal of neurology* 2007;254(9):1238-1243.
28 26 62. Funkiewiez A, Ardouin C, Caputo E, et al. Long term effects of bilateral subthalamic
29 27 nucleus stimulation on cognitive function, mood, and behaviour in Parkinson's disease.
30 28 *Journal of neurology, neurosurgery, and psychiatry* 2004;75(6):834-839.
31 29 63. Gervais-Bernard H, Xie-Brustolin J, Mertens P, et al. Bilateral subthalamic nucleus
32 30 stimulation in advanced Parkinson's disease: five year follow-up. *Journal of neurology*
33 31 2009;256(2):225-233.
34 32 64. Schupbach WM, Chastan N, Welter ML, et al. Stimulation of the subthalamic nucleus
35 33 in Parkinson's disease: a 5 year follow up. *Journal of neurology, neurosurgery, and psychiatry*
36 34 2005;76(12):1640-1644.
37 35 65. Temel Y, Visser-Vandewalle V, van der Wolf M, et al. Monopolar versus bipolar high
38 36 frequency stimulation in the rat subthalamic nucleus: differences in histological damage.
39 37 *Neuroscience letters* 2004;367(1):92-96.
40 38 66. Chaturvedi A, Lujan JL, McIntyre CC. Artificial neural network based
41 39 characterization of the volume of tissue activated during deep brain stimulation. *Journal of*
42 40 *neural engineering* 2013;10(5):056023.
43 41 67. Creed MC, Hamani C, Nobrega JN. Effects of repeated deep brain stimulation on
44 42 depressive- and anxiety-like behavior in rats: comparing entopeduncular and subthalamic
45 43 nuclei. *Brain Stimul* 2013;6(4):506-514.
46 44 68. Aleksandrova LR, Creed MC, Fletcher PJ, Lobo DS, Hamani C, Nobrega JN. Deep
47 45 brain stimulation of the subthalamic nucleus increases premature responding in a rat gambling
48 46 task. *Behavioural brain research* 2013;245:76-82.
49 47 69. Darbaky Y, Forni C, Amalric M, Baunez C. High frequency stimulation of the
50 48 subthalamic nucleus has beneficial antiparkinsonian effects on motor functions in rats, but
51 49 less efficiency in a choice reaction time task. *Eur J Neurosci* 2003;18(4):951-956.
52
53
54
55
56
57
58
59
60

STN-DBS and Apathy in Parkinson's Disease

- 1 70. Abulseoud OA, Kasasbeh A, Min HK, et al. Stimulation-Induced Transient Nonmotor
2 Psychiatric Symptoms following Subthalamic Deep Brain Stimulation in Patients with
3 Parkinson's Disease: Association with Clinical Outcomes and Neuroanatomical Correlates.
4 *Stereotactic and functional neurosurgery* 2016;94(2):93-101.
- 5 71. Simmons DB, Dashtipour K. The Quantitative Measurement of Reversible Acute
6 Depression after Subthalamic Deep Brain Stimulation in a Patient with Parkinson Disease.
7 *Case reports in neurological medicine* 2015;2015:165846.
- 8 72. Alkemade A, Schnitzler A, Forstmann BU. Topographic organization of the human
9 and non-human primate subthalamic nucleus. *Brain Struct Funct* 2015;220(6):3075-3086.
- 10 73. Lambert C, Zrinzo L, Nagy Z, et al. Confirmation of functional zones within the
11 human subthalamic nucleus: patterns of connectivity and sub-parcellation using diffusion
12 weighted imaging. *NeuroImage* 2012;60(1):83-94.
- 13 74. Mallet L, Schupbach M, N'Diaye K, et al. Stimulation of subterritories of the
14 subthalamic nucleus reveals its role in the integration of the emotional and motor aspects of
15 behavior. *Proc Natl Acad Sci U S A* 2007;104(25):10661-10666.
- 16 75. Stefurak T, Mikulis D, Mayberg H, et al. Deep brain stimulation for Parkinson's
17 disease dissociates mood and motor circuits: a functional MRI case study. *Movement
18 disorders : official journal of the Movement Disorder Society* 2003;18(12):1508-1516.
- 19 76. Roji R, Centeno C. The use of methylphenidate to relieve fatigue. *Current opinion in
20 supportive and palliative care* 2017;11(4):299-305.
- 21 77. Skorvanek M, Gdovinova Z, Rosenberger J, et al. The associations between fatigue,
22 apathy, and depression in Parkinson's disease. *Acta Neurol Scand* 2015;131(2):80-87.
- 23 78. Benazzouz A, Gao D, Ni Z, Benabid AL. High frequency stimulation of the STN
24 influences the activity of dopamine neurons in the rat. *Neuroreport* 2000;11(7):1593-1596.
- 25 79. Min HK, Ross EK, Jo HJ, et al. Dopamine Release in the Nonhuman Primate Caudate
26 and Putamen Depends upon Site of Stimulation in the Subthalamic Nucleus. *The Journal of
27 neuroscience : the official journal of the Society for Neuroscience* 2016;36(22):6022-6029.
- 28 80. Bruet N, Windels F, Bertrand A, Feuerstein C, Poupard A, Savasta M. High frequency
29 stimulation of the subthalamic nucleus increases the extracellular contents of striatal
30 dopamine in normal and partially dopaminergic denervated rats. *J Neuropathol Exp Neurol*
31 2001;60(1):15-24.
- 32 81. Kocobicak E, Jahanshahi A, Schonfeld L, Heschem SA, Temel Y, Tan S. Deep Brain
33 Stimulation of the Rat Subthalamic Nucleus Induced Inhibition of Median Raphe
34 Serotonergic and Dopaminergic Neurotransmission. *Turk Neurosurg* 2015;25(5):721-727.
- 35 82. Zhao XD, Cao YQ, Liu HH, Li FQ, You BM, Zhou XP. Long term high frequency
36 stimulation of STN increases dopamine in the corpus striatum of hemiparkinsonian rhesus
37 monkey. *Brain Res* 2009;1286:230-238.
- 38 83. Belin D, Everitt BJ. Cocaine seeking habits depend upon dopamine-dependent serial
39 connectivity linking the ventral with the dorsal striatum. *Neuron* 2008;57(3):432-441.
- 40 84. Haber SN, Knutson B. The reward circuit: linking primate anatomy and human
41 imaging. *Neuropsychopharmacology : official publication of the American College of
42 Neuropsychopharmacology* 2010;35(1):4-26.
- 43 85. Bjorklund A, Dunnett SB. Dopamine neuron systems in the brain: an update. *Trends
44 in neurosciences* 2007;30(5):194-202.
- 45 86. Gittis AH, Kreitzer AC. Striatal microcircuitry and movement disorders. *Trends in
46 neurosciences* 2012;35(9):557-564.
- 47 87. Sesack SR, Grace AA. Cortico-Basal Ganglia reward network: microcircuitry.
48 *Neuropsychopharmacology : official publication of the American College of
49 Neuropsychopharmacology* 2010;35(1):27-47.

STN-DBS and Apathy in Parkinson's Disease

- 1 88. Simon MJ, Higuera-Matas A, Roura-Martinez D, et al. Changes in D1 but not D2
2 dopamine or mu-opioid receptor expression in limbic and motor structures after lateral
3 hypothalamus electrical self-stimulation: A quantitative autoradiographic study. *Neurobiology*
4 *of learning and memory* 2016;127:17-26.
- 5 89. Le Foll B, Goldberg SR, Sokoloff P. The dopamine D3 receptor and drug dependence:
6 effects on reward or beyond? *Neuropharmacology* 2005;49(4):525-541.
- 7 90. Simpson EH, Winiger V, Biezonski DK, Haq I, Kandel ER, Kellendonk C. Selective
8 overexpression of dopamine D3 receptors in the striatum disrupts motivation but not
9 cognition. *Biol Psychiatry* 2014;76(10):823-831.
- 10 91. Drew MR, Simpson EH, Kellendonk C, et al. Transient overexpression of striatal D2
11 receptors impairs operant motivation and interval timing. *The Journal of neuroscience : the*
12 *official journal of the Society for Neuroscience* 2007;27(29):7731-7739.
- 13 92. Trifilieff P, Feng B, Urizar E, et al. Increasing dopamine D2 receptor expression in the
14 adult nucleus accumbens enhances motivation. *Mol Psychiatry* 2013;18(9):1025-1033.
- 15 93. Ishibashi K, Ishii K, Oda K, Mizusawa H, Ishiwata K. Binding of pramipexole to
16 extrastriatal dopamine D2/D3 receptors in the human brain: a positron emission tomography
17 study using 11C-FLB 457. *PloS one* 2011;6(3):e17723.
- 18 94. Knight EJ, Testini P, Min HK, et al. Motor and Nonmotor Circuitry Activation
19 Induced by Subthalamic Nucleus Deep Brain Stimulation in Patients With Parkinson Disease:
20 Intraoperative Functional Magnetic Resonance Imaging for Deep Brain Stimulation. *Mayo*
21 *Clinic proceedings* 2015;90(6):773-785.
- 22 95. Carriere N, Besson P, Dujardin K, et al. Apathy in Parkinson's disease is associated
23 with nucleus accumbens atrophy: a magnetic resonance imaging shape analysis. *Movement*
24 *disorders : official journal of the Movement Disorder Society* 2014;29(7):897-903.
- 25 96. Reijnders JS, Scholtissen B, Weber WE, Aalten P, Verhey FR, Leentjens AF.
26 Neuroanatomical correlates of apathy in Parkinson's disease: A magnetic resonance imaging
27 study using voxel-based morphometry. *Movement disorders : official journal of the*
28 *Movement Disorder Society* 2010;25(14):2318-2325.
- 29 97. Maillet A, Krack P, Lhommee E, et al. The prominent role of serotonergic
30 degeneration in apathy, anxiety and depression in de novo Parkinson's disease. *Brain : a*
31 *journal of neurology* 2016;139(Pt 9):2486-2502.
- 32 98. Creed MC, Hamani C, Bridgman A, Fletcher PJ, Nobrega JN. Contribution of
33 decreased serotonin release to the antidyskinetic effects of deep brain stimulation in a rodent
34 model of tardive dyskinesia: comparison of the subthalamic and entopeduncular nuclei. *The*
35 *Journal of neuroscience : the official journal of the Society for Neuroscience*
36 2012;32(28):9574-9581.
- 37 99. Chang WL, Breier MR, Yang A, Swerdlow NR. Disparate effects of pramipexole on
38 locomotor activity and sensorimotor gating in Sprague-Dawley rats. *Pharmacol Biochem*
39 *Behav* 2011;99(4):634-638.
- 40 100. Riddle JL, Rokosik SL, Napier TC. Pramipexole- and methamphetamine-induced
41 reward-mediated behavior in a rodent model of Parkinson's disease and controls. *Behavioural*
42 *brain research* 2012;233(1):15-23.
- 43 101. Favier M, Carcenac C, Drui G, et al. Implication of dorsostriatal D3 receptors in
44 motivational processes: a potential target for neuropsychiatric symptoms in Parkinson's
45 disease. *Sci Rep* 2017;7:41589.
- 46 102. Hurley MJ, Jenner P. What has been learnt from study of dopamine receptors in
47 Parkinson's disease? *Pharmacol Ther* 2006;111(3):715-728.
- 48 103. Marin-Lahoz J, Pagonabarraga J, Martinez-Horta S, et al. Parkinson's Disease:
49 Impulsivity Does Not Cause Impulse Control Disorders but Boosts Their Severity. *Frontiers*
50 *in psychiatry* 2018;9:465.

STN-DBS and Apathy in Parkinson's Disease

104. Weintraub D, Koester J, Potenza MN, et al. Impulse control disorders in Parkinson disease: a cross-sectional study of 3090 patients. *Arch Neurol* 2010;67(5):589-595.
105. Houeto JL, Magnard R, Dalley JW, Belin D, Carnicella S. Trait Impulsivity and Anhedonia: Two Gateways for the Development of Impulse Control Disorders in Parkinson's Disease? *Front Psychiatry* 2016;7:91.
106. Belin D, Mar AC, Dalley JW, Robbins TW, Everitt BJ. High impulsivity predicts the switch to compulsive cocaine-taking. *Science (New York, NY)* 2008;320(5881):1352-1355.
107. Scangos KW, Shahlaie K. Acute Frequency-Dependent Hypomania Induced by Ventral Subthalamic Nucleus Deep Brain Stimulation in Parkinson's Disease: A Case Report. *Biological psychiatry* 2017;82(5):e39-e41.
108. Welter ML, Schupbach M, Czernecki V, et al. Optimal target localization for subthalamic stimulation in patients with Parkinson disease. *Neurology* 2014;82(15):1352-1361.
109. Pelloux Y, Meffre J, Giorla E, Baunez C. The subthalamic nucleus keeps you high on emotion: behavioral consequences of its inactivation. *Frontiers in behavioral neuroscience* 2014;8:414.
110. Ardouin C, Voon V, Worbe Y, et al. Pathological gambling in Parkinson's disease improves on chronic subthalamic nucleus stimulation. *Movement disorders : official journal of the Movement Disorder Society* 2006;21(11):1941-1946.
111. Bandini F, Primavera A, Pizzorno M, Cocito L. Using STN DBS and medication reduction as a strategy to treat pathological gambling in Parkinson's disease. *Parkinsonism & related disorders* 2007;13(6):369-371.
112. Knobel D, Aybek S, Pollo C, Vingerhoets FJ, Berney A. Rapid resolution of dopamine dysregulation syndrome (DDS) after subthalamic DBS for Parkinson disease (PD): a case report. *Cognitive and behavioral neurology : official journal of the Society for Behavioral and Cognitive Neurology* 2008;21(3):187-189.
113. Witjas T, Baunez C, Henry JM, et al. Addiction in Parkinson's disease: impact of subthalamic nucleus deep brain stimulation. *Movement disorders : official journal of the Movement Disorder Society* 2005;20(8):1052-1055.
114. Castrioto A, Carnicella S, Fraix V, Chabardes S, Moro E, Krack P. Reversing dopaminergic sensitization. *Movement disorders : official journal of the Movement Disorder Society* 2017;32(12):1679-1683.
115. Pelloux Y, Baunez C. Deep brain stimulation for addiction: why the subthalamic nucleus should be favored. *Curr Opin Neurobiol* 2013;23(4):713-720.
116. Tan SK, Janssen ML, Jahanshahi A, et al. High frequency stimulation of the subthalamic nucleus increases c-fos immunoreactivity in the dorsal raphe nucleus and afferent brain regions. *J Psychiatr Res* 2011;45(10):1307-1315.
117. Martinez D, Gil R, Slifstein M, et al. Alcohol dependence is associated with blunted dopamine transmission in the ventral striatum. *Biological psychiatry* 2005;58(10):779-786.
118. Barak S, Carnicella S, Yowell QV, Ron D. Glial cell line-derived neurotrophic factor reverses alcohol-induced allostasis of the mesolimbic dopaminergic system: implications for alcohol reward and seeking. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2011;31(27):9885-9894.
119. Martinez D, Greene K, Broft A, et al. Lower level of endogenous dopamine in patients with cocaine dependence: findings from PET imaging of D(2)/D(3) receptors following acute dopamine depletion. *The American journal of psychiatry* 2009;166(10):1170-1177.
120. Le Moal M, Koob GF. Drug addiction: pathways to the disease and pathophysiological perspectives. *Eur Neuropsychopharmacol* 2007;17(6-7):377-393.
121. Paxinos G WC. *The rat brain in stereotaxic coordinates*. 1998.

STN-DBS and Apathy in Parkinson's Disease

1
2
3 1
4 2
5 3
6 4
7 5
8 **Figures legends**
9 6

10 7 **FIGURE 1:** Experimental design and electrode implantation.

11 8 **(A)** Time course of the study, groups of animals and histological controls

12 9 After learning the sucrose self-administration task, lesion and implantation of electrodes, rats
13 10 were divided into a stimulated and a non-stimulated group according to their performances,
14 11 to constitute initially equal performance groups. Then, after 4 days of subthalamic nucleus
15 12 deep brain stimulation (STN-DBS) with further sucrose self-administration, pramipexole
16 13 (PPX) or vehicle (Veh) was injected twice a day into half of the stimulated (Non lesioned rats:
17 14 STN-DBS + PPX, n = 8; STN-DBS + Veh, n = 13; lesioned rats: Lesion + STN-DBS + PPX, n
18 15 = 5; Lesion + STN-DBS + Veh, n = 5) and non-stimulated rats (Control + PPX, n = 22;
19 16 Control + Veh, n = 22; Lesioned rats: Lesion + PPX, n = 4; Lesion + Veh, n = 6) for 20 days.
20 17 During this time, sucrose self-administration was continued for several days and then
21 18 followed successively by other behavioral tests: the two-bottle choice test, the stepping test
22 19 and finally the open area test. **(B)** Positions of bilateral DBS electrode tips in left and right
23 20 STNs of stimulated animals in representative coronal sections (anteroposterior relative to
24 21 Bregma: -3.60 mm; -3.80 mm; -4.16 mm), reproduced from Paxinos and Watson.¹²¹ Each bar
25 22 represents the placement of one electrode. **(C)** and **(D)** Example of electrode trace within the
26 23 left STN stimulated at 100 μ A **(C)** or 225 μ A **(D)**, after Cresyl violet staining. Scale bar: 1 mm.
27 24 IC, Internal Capsule; LH, Lateral Hypothalamus; ZI, Zona Incerta.

28 25 **(E)** and **(F)** Representative photomicrographs of coronal sections stained for tyrosine
29 26 hydroxylase (TH) in striatal (bottom, 1.6 mm to bregma) and mesencephalic (top, -5.6 mm to
30 27 bregma) regions from naïve (top) and lesioned (bottom) rats.¹²¹ Scale bar = 1 mm. **(G, H)**
31 28 Quantification of TH ImmunoReactivity (IR) loss in the midbrain **(G)** and the striatum **(H)**,
32 29 expressed as the percentage difference compared to the mean value obtained for sham
33 30 operated animals. The SNc bilateral lesion produced an important but partial denervation of

STN-DBS and Apathy in Parkinson's Disease

1 the dorsal striatum. The ventral tegmental area and the nucleus accumbens were preserved
 2 from the lesion. Control + Veh: n = 22; Lesion: n = 20. Control + Veh versus Lesion: * $p < .05$;
 3 *** $p < .001$. DS: dorsal striatum; NAc: nucleus accumbens; SNc: substantia nigra compacta,
 4 VTA: ventral tegmental area.

5
 6 **FIGURE 2:** Subthalamic nucleus deep brain stimulation (STN-DBS) induces a deficit of
 7 preparatory behaviors in 2.5% sucrose self-administration that is rescued by pramipexole
 8 (PPX). **(A)** Time course of the sucrose self-administration experiment. STN-DBS induced a
 9 significant decrease of the number of sucrose deliveries. PPX induced a total reversion of
 10 this deficit. **(B)** Mean sucrose deliveries over the last 3 days of experiment. **(C)** STN-DBS
 11 and PPX did not alter the preference for sucrose.
 12 Control + Veh: n = 22; Control + PPX: n = 22; STN-DBS + Veh: n = 13; STN-DBS + PPX: n =
 13 8. Data shown as means \pm SEM. Control + Veh versus STN-DBS + Veh: * $p < .05$; ** $p < .01$;
 14 STN-DBS + Veh versus STN-DBS + PPX: # $p < .05$; ## $p < .01$.

15
 16 **FIGURE 3:** Subthalamic nucleus deep brain stimulation (STN-DBS) and pramipexole (PPX)
 17 promote hypo and hyper locomotor activity respectively but do not impair motor abilities. **(A)**
 18 Total distance travelled during a 1h open area test. STN-DBS induced a non-significant
 19 tendency towards decreased basal locomotor activity that was counteracted by treatment
 20 with PPX. In control rats, PPX induced a dramatic increase in the distance traveled. **(B)** STN-
 21 DBS and PPX did not change the mean ambulatory speed during the last 2 periods of the
 22 open area test. **(C)** STN-DBS and/or PPX did not alter the fine motor skills of front limbs as
 23 demonstrated by adjusting steps in the course of the stepping test. Control + Veh: n = 22;
 24 Control + PPX: n = 22; STN-DBS + Veh: n = 6; STN-DBS + PPX: n = 8. Data shown as
 25 means \pm SEM. Control + PPX versus Control + Veh: * $p < .05$.

STN-DBS and Apathy in Parkinson's Disease

1 **FIGURE 4:** STN-DBS exacerbates the deficit of preparatory behaviors of the existing
2 motivational deficit in a rodent model of the neuropsychiatric symptoms of PD. **(A)** Time
3 course of the sucrose self-administration experiment. STN-DBS decreased the number of
4 sucrose deliveries. PPX induced a complete reversion of this deficit. **(B)** Mean sucrose
5 deliveries over the last 5 days of experiment. **(C)** STN-DBS and PPX did not alter the
6 preference for sucrose in 6-OHDA rats. Lesion + Veh: n = 6; Lesion + PPX: n = 4; Lesion +
7 STN-DBS + Veh: n = 5; Lesion + STN-DBS + PPX: n = 5. Data shown as means \pm SEM.
8 Lesion + Veh versus Lesion + STN-DBS + Veh: $*p < .05$; Lesion + STN-DBS + Veh versus
9 Lesion + STN-DBS + PPX: $\#p < .05$.

10
11 **FIGURE 5:** Subthalamic nucleus deep brain stimulation (STN-DBS) or pramipexole (PPX)
12 individually promote hypoactivity but do not impair motor abilities. **(A)** Total distance traveled
13 during a 1h open area test. STN-DBS induced a non-significant tendency towards decreased
14 basal locomotor activity. In non-stimulated rats, PPX induced a dramatic decrease in the
15 distance traveled. The combination of both treatments increased activity. **(B)** STN-DBS and
16 PPX did not change the mean ambulatory speed during the open area test. **(C)** STN-DBS
17 and/or PPX did not alter the fine motor skills of front limbs as demonstrated by adjusting
18 steps in the course of the stepping test. Lesion + Veh: n = 6; Lesion + PPX: n = 4; Lesion +
19 STN-DBS + Veh: n = 5; Lesion + STN-DBS + PPX: n = 5. Data shown as means \pm SEM.
20 Lesion + PPX versus Lesion + STN-DBS + PPX: $*p < .05$.

Figure 1

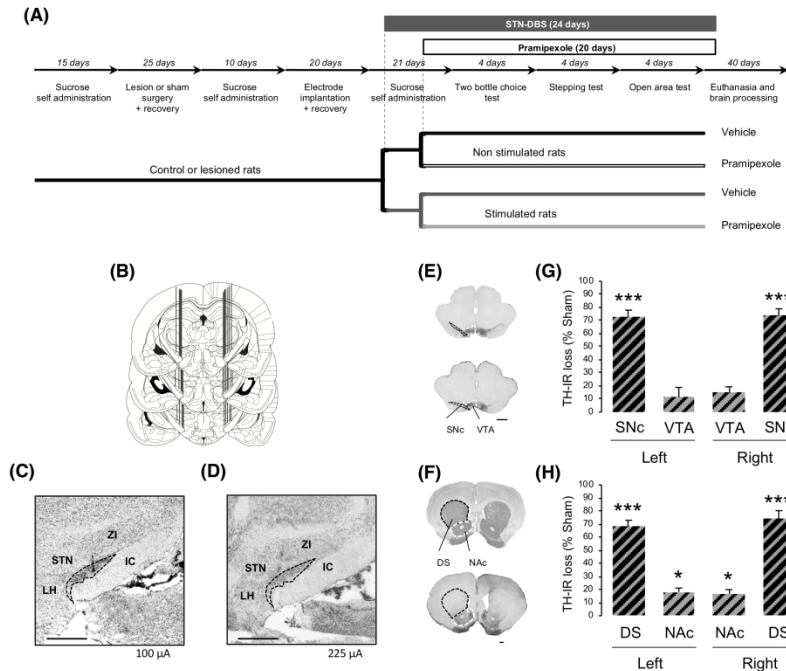


FIGURE 1: Experimental design and electrode implantation.

(A) Time course of the study, groups of animals and histological controls. After learning the sucrose self-administration task, lesion and implantation of electrodes, rats were divided into a stimulated and a non-stimulated group according to their performances, to constitute initially equal performance groups. Then, after 4 days of subthalamic nucleus deep brain stimulation (STN-DBS) with further sucrose self-administration, pramipexole (PPX) or vehicle (Veh) was injected twice a day into half of the stimulated (Non lesioned rats: STN-DBS + PPX, $n = 8$; STN-DBS + Veh, $n = 13$; lesioned rats: Lesion + STN-DBS + PPX, $n = 5$; Lesion + STN-DBS + Veh, $n = 5$) and non-stimulated rats (Control + PPX, $n = 22$; Control + Veh, $n = 22$; Lesioned rats: Lesion + PPX, $n = 4$; Lesion + Veh, $n = 6$) for 20 days. During this time, sucrose self-administration was continued for several days and then followed successively by other behavioral tests: the two-bottle choice test, the stepping test and finally the open area test. (B) Positions of bilateral DBS electrode tips in left and right STNs of stimulated animals in representative coronal sections (anteroposterior relative to Bregma: -3.60 mm; -3.80 mm; -4.16 mm), reproduced from Paxinos and Watson.¹¹⁹ Each bar represents the placement of one electrode. (C) and (D) Example of electrode trace

1
2
3 within the left STN stimulated at 100 μ A (C) or 225 μ A (D), after Cresyl violet staining. Scale bar: 1 mm. IC,
4 Internal Capsule; LH, Lateral Hypothalamus; ZI, Zona Incerta.

5 (E) and (F) Representative photomicrographs of coronal sections stained for tyrosine hydroxylase (TH) in
6 striatal (bottom, 1.6 mm to bregma) and mesencephalic (top, -5.6 mm to bregma) regions from naïve (top)
7 and lesioned (bottom) rats.119 Scale bar = 1 mm. (G, H) Quantification of TH Immuno Reactivity (IR) loss
8 in the midbrain (G) and the striatum (H), expressed as percentage difference in the mean value obtained for
9 sham operated animals. The SNc bilateral lesion produced an important but partial denervation of the dorsal
10 striatum. The ventral tegmental area and the nucleus accumbens were preserved from the lesion. Control +
11 Veh: n = 22; Lesion: n = 20. Control + Veh versus Lesion: *p < .05; ***p < .001. DS: dorsal striatum;
12 NAc: nucleus accumbens; SNc: substantia nigra compacta, VTA: ventral tegmental area.

13
14 190x275mm (300 x 300 DPI)
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Figure 2

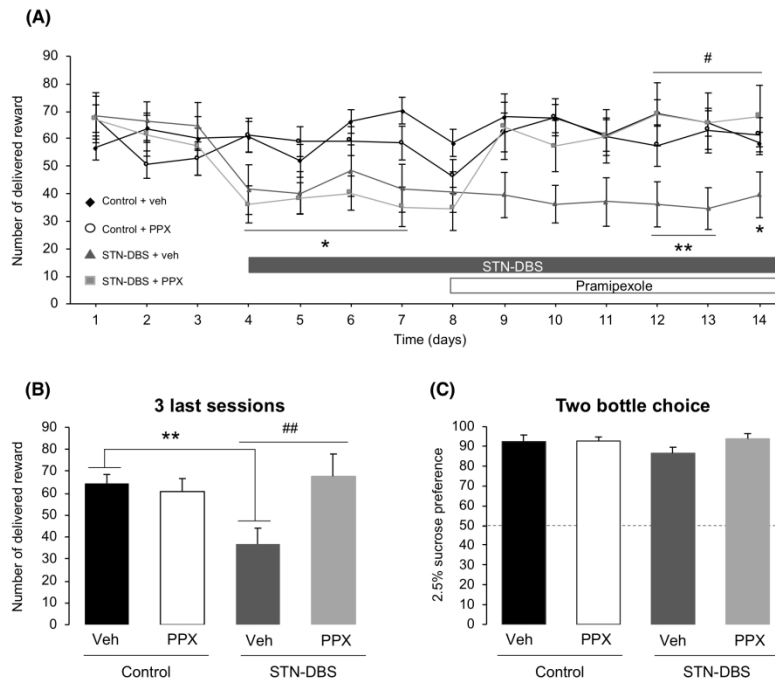


FIGURE 2: Subthalamic nucleus deep brain stimulation (STN-DBS) induces a deficit of preparatory behaviors in 2.5% sucrose self-administration that is rescued by pramipexole (PPX). (A) Time course of the sucrose self-administration experiment. STN-DBS induced a significant decrease of the number of sucrose deliveries. Pramipexole induced a total reversion of this deficit. (B) Mean sucrose deliveries over the last 3 days of experiment. (C) Subthalamic nucleus deep brain stimulation (STN-DBS) and pramipexole (PPX) do not alter the preference for sucrose.

Control + Veh: $n = 22$; Control + PPX: $n = 22$; STN-DBS + Veh: $n = 13$; STN-DBS + PPX: $n = 8$. Data shown as means \pm SEM. Control + Veh versus STN-DBS + Veh: $*p < .05$; $**p < .01$; STN-DBS + Veh versus STN-DBS + PPX: $\#p < .05$; $\#\#p < .01$.

190x275mm (300 x 300 DPI)

Figure 3

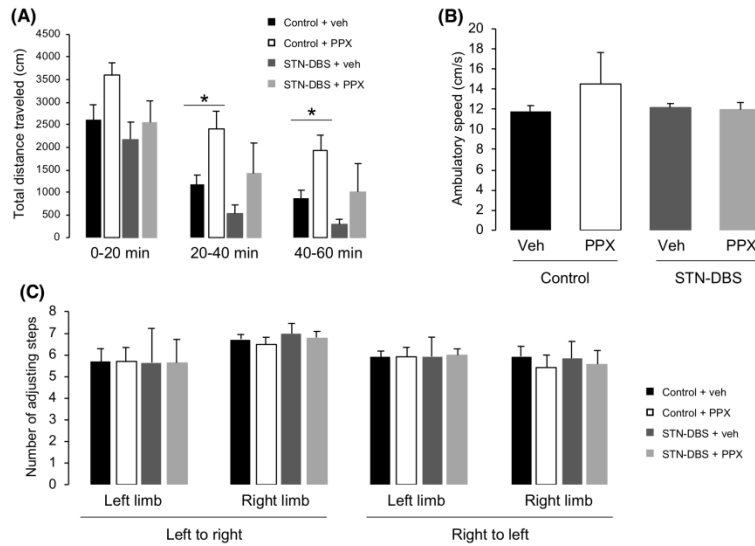


FIGURE 3: Subthalamic nucleus deep brain stimulation (STN-DBS) and pramipexole (PPX) promote hypo and hyper locomotor activity respectively but do not impair motor abilities. (A) Total distance travelled during a 1h open area test. STN-DBS induced a non-significant tendency towards decreased basal locomotor activity that was counteracted by treatment with pramipexole. In control rats, pramipexole induced a dramatic increase in the distance traveled. (B) STN-DBS and pramipexole did not change the mean ambulatory speed during the last 2 periods of the open area test. (C) STN-DBS and/or pramipexole did not alter the fine motor skills of front limbs as demonstrated by adjusting steps in the course of the stepping test. Control + Veh: n = 22; Control + PPX: n = 22; STN-DBS + Veh: n = 6; STN-DBS + PPX: n = 8. Data shown as means \pm SEM. Control + PPX versus Control + Veh: *p < .05.

190x275mm (300 x 300 DPI)

Figure 4

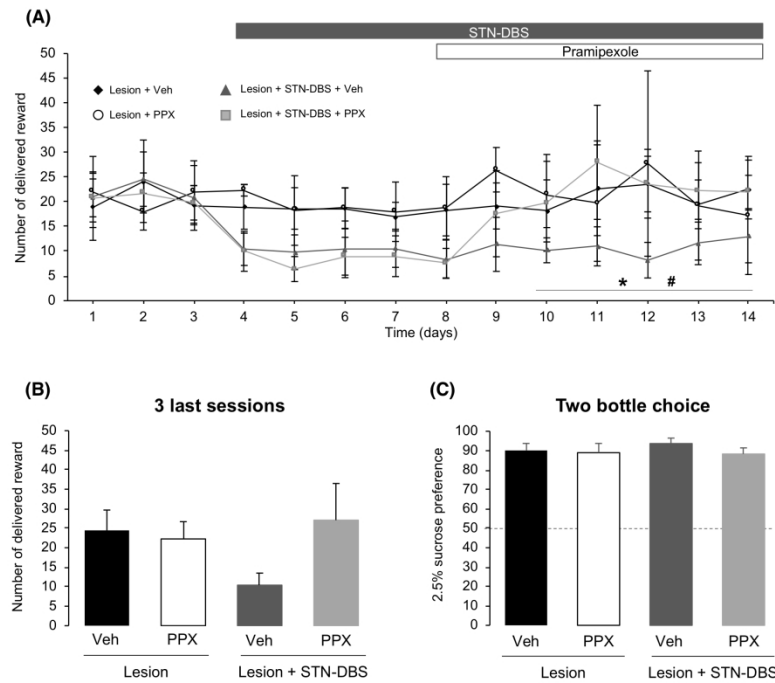


FIGURE 4: STN-DBS exacerbates the deficit of preparatory behaviors of the existing motivational deficit in a rodent model of the neuropsychiatric symptoms of PD. (A) Time course of the sucrose self-administration experiment. STN-DBS induced a decrease of the number of sucrose deliveries. Pramipexole induced a complete reversion of this deficit. (B) Mean sucrose deliveries over the last 5 days of experiment. (C) Subthalamic nucleus deep brain stimulation (STN-DBS) and pramipexole (PPX) do not alter the preference for sucrose in 6-OHDA rats. Lesion + Veh: $n = 6$; Lesion + PPX: $n = 4$; Lesion + STN-DBS + Veh: $n = 5$; Lesion + STN-DBS + PPX: $n = 5$. Data shown as means \pm SEM. Lesion + Veh versus Lesion + STN-DBS + Veh: $*p < .05$; Lesion + STN-DBS + Veh versus Lesion + STN-DBS + PPX: $\#p < .05$.

190x275mm (300 x 300 DPI)

Figure 5

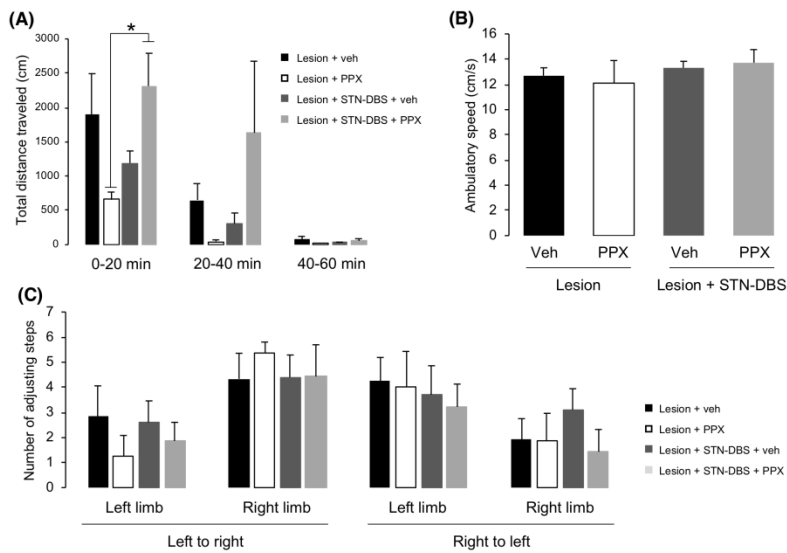


FIGURE 5: Subthalamic nucleus deep brain stimulation (STN-DBS) and pramipexole (PPX) individually promotes hypoactivity but do not impair motor abilities. (A) Total distance travelled during a 1h open area test. STN-DBS induced a non-significant tendency towards decreased basal locomotor activity. In non-stimulated rats, pramipexole induced a dramatic decrease in the distance traveled. The combination of both treatments increased activity. (B) STN-DBS and pramipexole did not change the mean ambulatory speed during the open area test. (C) STN-DBS and/or pramipexole did not alter the fine motor skills of front limbs as demonstrated by adjusting steps in the course of the stepping test. Lesion + Veh: n = 6; Lesion + PPX: n = 4; Lesion + STN-DBS + Veh: n = 5; Lesion + STN-DBS + PPX: n = 5. Data shown as means \pm SEM. Lesion + PPX versus Lesion + STN-DBS + PPX: *p < .05.

190x275mm (300 x 300 DPI)

1 Subthalamic nucleus stimulation impairs motivation: implication for apathy in 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

Parkinson's disease

Yvan Vachez, PhD^{1,2}, Carole Carcenac, PhD^{1,2}, Robin Magnard, PhD^{1,2}, Lydia Kerkerian -Le Goff, PhD³, Pascal Salin, PhD³, Marc Savasta, PhD^{1,2}, Sebastien Carnicella, PhD^{1,2} and Sabrina Boulet, PhD^{1,2}

Affiliations :

¹Inserm, U1216, F- 38000 Grenoble, France.

²Univ. Grenoble Alpes, Grenoble Institut des Neurosciences, GIN, F-38000 Grenoble, France.

³Aix Marseille Univ, CNRS, IBDM, Marseille, France.

Number of words: 1331

Supplemental Material and Methods

Bilateral 6-OHDA lesions

As previously described,¹⁻⁴ rats were first treated with desipramine hydrochloride (25 mg.kg⁻¹ in 0.9% NaCl, subcutaneously; Sigma; St Quentin-Fallavier, France) to protect noradrenergic neurons 30 min before 6-OHDA injection.⁵ They were then anesthetized with a mixture of xylazine (15 mg.kg⁻¹, i.p) and ketamine (100 mg.kg⁻¹, i.p) and secured in a Kopf stereotaxic apparatus (Phymep, Paris, France). As previously described², 6 µg of 6-OHDA dissolved in 2.3 µl sterile 0.9% NaCl with 0.2% ascorbic acid (Sigma), or 2.3 µl of vehicle (0.9% NaCl, 0.02% ascorbic acid) were injected bilaterally, at a flow rate of 0.5 µl min⁻¹. The stereotaxic coordinates of the injection site relative to bregma according to the stereotaxic atlas of Paxinos and Watson⁶ were as follows: anteroposterior (AP), -5.4 mm; mediolateral (L), ±1.8 mm, and dorsoventral (DV), -8.1 mm; with the incisor bar at +3.2 mm below the interaural plane. The cannulae were left in position for 5 min after each injection to allow absorption of the solution without spreading. After recovery from anesthesia, the animals were returned to the facility for 3 weeks, to allow the 6-OHDA lesion to develop and stabilize.

Bilateral implantation of electrodes in the subthalamic nucleus

Rats were bilaterally implanted with monopolar electrodes consisting of platinum-iridium wire insulated with Teflon with a 400 μm exposed end (wire diameter, 110 μm insulated, 76 μm bare, PT-IR Teflon, Phymep, Paris, France). Stereotaxic coordinates (relative to bregma, according to the stereotaxic atlas of Paxinos and Watson⁶) were: AP, -3.8 mm; L, ± 2.4 mm, and DV, -7.8 mm; with the incisor bar at +3.2 mm below the interaural plane. The exposed end of the electrode, located in the STN, corresponded to the negative stimulation pole. A screw (Phymep, Paris, France, 0-80x1/16) fixed on the skull was used as the positive pole. Electrodes were soldered to corresponding contacts of the microstimulator support (ISENUSTIMV7, ISEN, Toulon, France) which was permanently fixed to the rat skull with dental cement (Superbond, Phymep, Paris, France). After recovery from anesthesia, animals were returned to the facility for 3 days, to allow recovery before the beginning of the behavioral experiments.

Subthalamic nucleus deep brain stimulation

Chronic long-lasting bilateral STN-DBS was performed using an electrical portable microstimulator system already validated in freely moving rats.⁷ This system has the advantages of leaving the animals free to move during behavioral tasks, of being removable and of allowing rapid and easy activation (ON/OFF), modulation of DBS parameters or battery change without strain of rats or anesthesia. Monopolar rather than bipolar electrodes were utilized because the former is the most widely applied in PD patients⁸ as well as in studies describing apathy under STN-DBS.⁹⁻¹⁵ Furthermore, a preclinical study in rat has clearly demonstrated that it reduces tissue damage compared to bipolar stimulation.¹⁶ The interface between electrodes and the microstimulator is a support with the top side designed as a platform to receive a microstimulator's plug in. The microstimulator is made up with classical structures allowing to regulate the frequency and the duty cycle. The power supply of the microstimulator consists of two 3 V flat lithium watch batteries (CR1220) connected in series.

1
2
3 1 The frequency and pulse width used, 130 Hz and 60 μ s respectively, were similar to those
4
5 2 applied in humans. For each animal, the intensity was gradually increased until dyskinetic
6
7 3 movement of the contralateral forelimb appeared as previously described¹⁷ and then
8
9 4 adjusted just below this pro-dyskinetic threshold. This value was conserved throughout the
10
11 5 study. The stimulation intensity was 183 \pm 13 μ A on average and ranged between 100 and
12
13
14 6 225 μ A.
15
16 7

8 ***Behavioral procedures***

9 Rats were not food or water deprived during the experimental procedures.

10 *Sucrose self-administration*

11 Rats were trained to work for 2.5% sucrose reward, chosen for its relatively moderate
12
13 rewarding outcome, in a self-administration task in operant chambers (Med Associates, St
14
15 Albans, VT, USA) under a fixed ratio 1 reinforcement schedule. Rats were given the choice
16
17 between two levers: an active and reinforced one, delivering 0.2 ml sucrose solution in a
18
19 receptacle when pressed, and an inactive, non-reinforced lever, producing nothing¹⁻⁴.

20 *Two bottle choice*

21 In their home cage, rats were given continuous access to two graduated 250 ml plastic
22
23 bottles (Techniplast, Lyon, France), for 3 days. One bottle contained tap water, whereas the
24
25 other contained 2.5% sucrose (Sigma) in tap water. Rats and bottles were weighed daily,
26
27 with the position of the bottles (left or right) alternated to control for side preference. The first
28
29 day was for acclimatation. The volumes of sucrose solution and water consumed on the
30
31 second and third days were averaged to determine preference for sucrose over water
32
33 (sucrose intake/total intake, expressed as a percentage).
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 *Stepping test*

2 Animals were moved sideways along a smooth-surfaced table over 90 cm and the number of
3 forelimb adjusting steps measured. The test was carried out three times for each paw.

5 *Open area*

6 Rats were placed in a dimly lit white Perspex (Castorama, Saint Martin d'Hères, France)
7 open arena (50 x 25 x 40cm) and horizontal distances traveled were recorded with a video-
8 tracking system to assess locomotor and basal activity (Viewpoint S.A., Champagne au Mont
9 d'Or, France), over a 1h period.

11 *Histological analysis*

12 Briefly, rats were sacrificed under chloral hydrate anesthesia at the end of the behavioral
13 experiments, intracardially perfused with NaCl (0.9%) and their brains frozen in cooled
14 isopentane (-40°C) and stored at -30°C. Serial coronal sections (14 µm-thick) of striatum,
15 mesencephalon and subthalamic nucleus were cut with a cryostat (Microm HM 500, Microm,
16 Francheville, France), collected on slides and stored at -30°C.

18 *Immunohistochemistry:*

19 Immunohistochemical analysis was performed as previously described.^{2, 17} 14 µm-thick
20 coronal sections from the striatum and the mesencephalon were incubated with an anti-TH
21 antibody, and then with a biotinylated goat anti-mouse IgG antibody. Immunoreactivity was
22 visualized with avidin-peroxidase conjugate.

24 *Quantification of the extent of the striatal DA denervation*

25 As described previously,² TH immunoreactivity (TH-IR) was quantified with the ICS
26 FrameWork computerized image analysis system (TRIBVN, 2.9.2 version, Châtillon, France)
27 coupled to a light microscope (Nikon, Eclipse 80i) and a Pike F-421C camera (ALLIED Vision
28 Technologies, Stadroda, Germany) for digitalization of the DS and the NAc (+ 0.7 to 2.2 mm

1 anterior to bregma), and of the SNc and the ventral tegmental area (VTA), (-5.6 mm to -4.8
2 mm). For all measurements, masks from these different striatal and mesencephalic sub-
3 regions were drawn with the computer analysis system to ensure that appropriate
4 comparisons were made between homologous anatomical regions. For each striatal sub-
5 region, optical densities (OD) were measured and averaged. The OD value obtained for an
6 unlabeled area (the corpus callosum) was used as the background and was subtracted from
7 each of the OD values measured. OD were expressed as percentages relative to the mean
8 optical density values obtained for the homologous regions of the sham-operated animals.

10 *Histological control of electrodes implantation*

11 Sections through the subthalamic nucleus were stained with Cresyl violet and analyzed
12 under a light microscope (Nikon, Eclipse 80i, TRIBVN, Châtillon, France) coupled to the ICS
13 FrameWork computerized image analysis system (TRIBVN, 2.9.2 version, Châtillon, France)
14 in order to check the positions of electrodes. Animals with incorrect electrode locations were
15 excluded from the study.

17 **Supplemental Figure and Figure Legend:**

18 **FIGURE S1:** Example of electrode trace and tissue state in animals stimulated at 100 μ A **(A)**
19 or 225 μ A **(B)**, after Cresyl violet staining. Scale bar: 1 mm.

21 **FIGURE S2:** Comparison of the effects of STN-DBS **(A)** or PPX treatment **(B)** in control and
22 lesioned animals on the mean sucrose deliveries over the last 3 days of sucrose self-
23 administration experiment. Data shown as means \pm SEM. **(A)** Control Sham: n = 22; Control
24 STN-DBS: n = 13; Lesion Sham n = 6; Lesion STN-DBS: n = 5. Control Sham versus Control
25 STN-DBS: *** $p < .001$; Control Sham versus Lesion sham: ### $p < .001$; Control STN-DBS
26 versus Lesion STN-DBS: ## $p < .01$. **(B)** Control Veh: n = 22; Control PPX: n = 22; Lesion

1
2
3 1 Veh: n = 6; Lesion PPX: n = 5. Control Veh versus Lesion Veh: $###p < .001$; Control PPX
4
5 2 versus Lesion PPX: $##p < .01$.
6
7 3
8
9 4
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

1. Carnicella S, Drui G, Boulet S, et al. Implication of dopamine D3 receptor activation in the reversion of Parkinson's disease-related motivational deficits. *Translational psychiatry* 2014;4:e401.
2. Drui G, Carnicella S, Carcenac C, et al. Loss of dopaminergic nigrostriatal neurons accounts for the motivational and affective deficits in Parkinson's disease. *Molecular psychiatry* 2014;19(3):358-367.
3. Favier M, Carcenac C, Drui G, et al. Implication of dorsostriatal D3 receptors in motivational processes: a potential target for neuropsychiatric symptoms in Parkinson's disease. *Scientific reports* 2017;7:41589.
4. Favier M, Duran T, Carcenac C, Drui G, Savasta M, Carnicella S. Pramipexole reverses Parkinson's disease-related motivational deficits in rats. *Movement disorders : official journal of the Movement Disorder Society* 2014;29(7):912-920.
5. Schwarting RK, Huston JP. Unilateral 6-hydroxydopamine lesions of meso-striatal dopamine neurons and their physiological sequelae. *Progress in neurobiology* 1996;49(3):215-266.
6. Paxinos G WC. *The rat brain in stereotaxic coordinates*. 1998.
7. Forni C, Mainard O, Melon C, Goguenheim D, Kerkerian-Le Goff L, Salin P. Portable microstimulator for chronic deep brain stimulation in freely moving rats. *Journal of neuroscience methods* 2012;209(1):50-57.
8. Benabid AL, Chabardes S, Mitrofanis J, Pollak P. Deep brain stimulation of the subthalamic nucleus for the treatment of Parkinson's disease. *The Lancet Neurology* 2009;8(1):67-81.
9. Drapier D, Drapier S, Sauleau P, et al. Does subthalamic nucleus stimulation induce apathy in Parkinson's disease? *Journal of neurology* 2006;253(8):1083-1091.
10. Thobois S, Ardouin C, Lhomme E, et al. Non-motor dopamine withdrawal syndrome after surgery for Parkinson's disease: predictors and underlying mesolimbic denervation. *Brain : a journal of neurology* 2010;133(Pt 4):1111-1127.
11. Castelli L, Lanotte M, Zibetti M, et al. Apathy and verbal fluency in STN-stimulated PD patients. An observational follow-up study. *Journal of neurology* 2007;254(9):1238-1243.
12. Castelli L, Perozzo P, Zibetti M, et al. Chronic deep brain stimulation of the subthalamic nucleus for Parkinson's disease: effects on cognition, mood, anxiety and personality traits. *European neurology* 2006;55(3):136-144.
13. Funkiewiez A, Ardouin C, Caputo E, et al. Long term effects of bilateral subthalamic nucleus stimulation on cognitive function, mood, and behaviour in Parkinson's disease. *Journal of neurology, neurosurgery, and psychiatry* 2004;75(6):834-839.
14. Gervais-Bernard H, Xie-Brustolin J, Mertens P, et al. Bilateral subthalamic nucleus stimulation in advanced Parkinson's disease: five year follow-up. *Journal of neurology* 2009;256(2):225-233.
15. Schupbach WM, Chastan N, Welter ML, et al. Stimulation of the subthalamic nucleus in Parkinson's disease: a 5 year follow up. *Journal of neurology, neurosurgery, and psychiatry* 2005;76(12):1640-1644.
16. Temel Y, Visser-Vandewalle V, van der Wolf M, et al. Monopolar versus bipolar high frequency stimulation in the rat subthalamic nucleus: differences in histological damage. *Neuroscience letters* 2004;367(1):92-96.
17. Boulet S, Lacombe E, Carcenac C, et al. Subthalamic stimulation-induced forelimb dyskinesias are linked to an increase in glutamate levels in the substantia nigra pars reticulata. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2006;26(42):10768-10776.

Supplemental figure 1

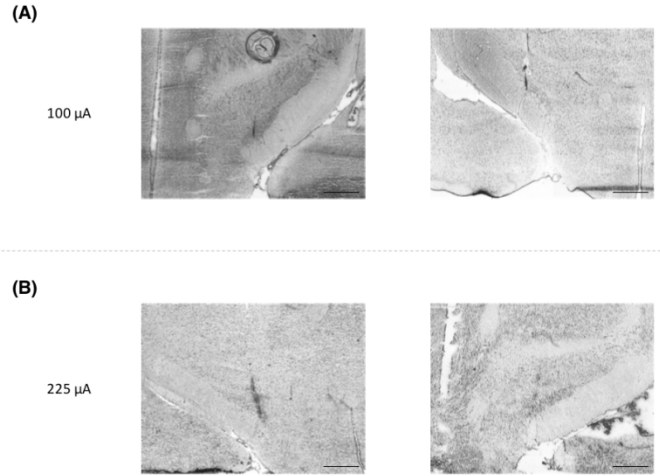


FIGURE S1: Example of electrode trace and tissue state in animals stimulated at 100 μ A (A) or 225 μ A (B), after Cresyl violet staining. Scale bar: 1 mm.

190x275mm (150 x 150 DPI)

Supplemental figure 2

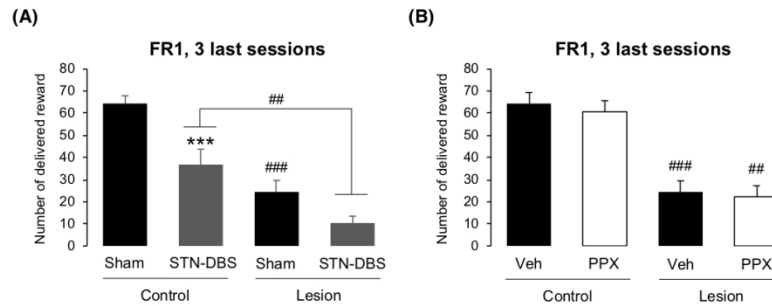


FIGURE S2: Comparison of the effects of STN-DBS (A) or PPX treatment (B) in control and lesioned animals on the mean sucrose deliveries over the last 3 days of sucrose self-administration experiment. Data shown as means \pm SEM. (A) Control Sham: $n = 22$; Control STN-DBS: $n = 13$; Lesion Sham $n = 6$; Lesion STN-DBS: $n = 5$. Control Sham versus Control STN-DBS: $***p < .001$; Control Sham versus Lesion sham: $###p < .001$; Control STN-DBS versus Lesion STN-DBS: $##p < .01$. (B) Control Veh: $n = 22$; Control PPX: $n = 22$; Lesion Veh: $n = 6$; Lesion PPX: $n = 5$. Control Veh versus Lesion Veh: $###p < .001$; Control PPX versus Lesion PPX: $##p < .01$.

190x275mm (150 x 150 DPI)